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Research Article

Evaluation of the effectiveness of pharmacotherapy for brain and heart diseases by monitoring the effects of drugs

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Abstract

Introduction: The study aims at analyzing the possibility of using a method of monitoring the action of drugs in real-time to assess the effectiveness of pharmacotherapy for brain and heart lesions.

Materials and methods: To assess the effect of drugs in the experiment and in the clinic, the temperature difference between the biologically active point and the intact skin zone was recorded every second for 2 minutes. The work involved experimental and clinical parts. The experimental study was performed on 81 rabbits. In the experiment, the effect of Meldonium was evaluated when treating doxorubicin cardiomyopathy and the effect of Cortexin – when treating experimental brain ischemia. The clinical testing of the method involved 10 healthy volunteers and 20 patients of both sexes, diagnosed with acute cerebrovascular events of the ischemic-stroke type.

Results and discussions: An increase of some indicators of differential thermometry of the biologically active point by 60% or more on the 7th day of the treatment concerning the values obtained before the treatment is indicative of a high probability of pronounced positive dynamics in the treatment of doxorubicin cardiomyopathy. If on the 7th day of the treatment, some indicators of differential thermometry of biologically active point C7 to exceed by 20% or more the similar indicators before the treatment, a high probability of pronounced positive dynamics in the treatment of ischemic stroke can be inferred.

Conclusion: A safe, non-invasive method for monitoring the effects of drugs in real-time, which does not require any special training of a doctor, has been developed.

Keywords

Meldonium, Cortexin, ischemic stroke, doxorubicin cardiomyopathy.

Introduction

Lesions of the central nervous system and cardiovascular system, as situations that affect the most important aspects of life, require a particularly accurate and rapid selection of drugs and their doses, as well as continuous monitoring of pharmacotherapy to be able to promptly change the treatment tactics.

The major problem in the structure of diseases of the central nervous system is cerebrovascular pathology and,

as its most severe manifestation – ischemic stroke due to a significant frequency of its development, a high percentage of disability and mortality among middle-aged and elderly people (Jungehilsing and Matthias 2017; Avrov et al. 2019). In turn, currently one of the main problems of cardiology is chronic heart failure, due to its significant prevalence and adverse outcomes (Kirichenko and Ebzeeva 2019). Among the etiological factors of chronic heart failure, cardiomyopathies and cardiomyopathy syndromes play a significant role in the structure of multi-organ pathology (Maron 2010). Despite the relatively low prevalence of cardiomyopathies, the medical and social significance of this group of diseases is quite high, due to the lack of clear diagnostic criteria and as well as poor prognosis of patient survival as a result of late diagnosis and inadequate and uncontrolled treatment. Therefore, the rationality and effectiveness of personalized pharmacotherapy in patients with ischemic stroke and cardiomyopathy are becoming a very important issue.

Nowadays, there are a large number of different tools and methods used to diagnose diseases and control therapies. Among them is an assessment of the dynamics of the patient's subjective perceptions and the dynamics of symptoms of the disease obtained by additional methods of the examination (Fedotov and Akulov 2013). However, each method individually and independently assesses the results of the treatment, determining the elimination of certain symptoms and normalization of instrumental and laboratory indicators, which cannot be considered as an assessment of the effectiveness of treatment and recovery of the patient (Ruksin 2001). The majority of the used methods record only significant changes in the structure and function of organs and systems. In turn, the constancy of structure and function is supported by a system of regulatory processes (Reznikov and Borisova 2012). Since the disruption of regulatory processes shows before disruptions of structures and functions, it would be logical to use methods that allow assessing these disruptions (Reznikov and Borisova 2012; Chang 2013).

Such methods may include assessing the state of internal organs by electrical or temperature parameters of biologically active points (Chang 2010; Samokhin and Gotovskiy 2012; Tabeeva 2016), in particular, by performing electro- and thermopuncture monitoring of the effect of drugs (Balashov 2004; Borisova et al. 2015). These data present an argument for a detailed study of the possibility of using thermopuncture diagnostics to assess the effect of drugs in real-time to optimize a treatment, which was implemented in the present study.

Materials and methods

This paper reports on the experimental and clinical study of the possibility of using a method for monitoring the action of drugs based on a mathematical analysis of rhythmic fluctuations in the temperature difference between the biologically active point (BAP) and the intact skin zone (a method of differential thermometry of biologically active points) to improve the effectiveness of pharmacotherapy for brain and heart diseases.

The experimental study was performed on 81 mature male Chinchilla rabbits, weighing 2.8–4.2 kg each, in compliance with the regulations of working with animals and according to the guidelines for experimental studies edited by Karkishchenko (2010) and Pokrovsky (2011). Euthanasia of animals was performed under mandatory ether anesthesia.

The experiment consisted of 2 stages.

The first stage of the experiment was conducted to identify the possibility of using the method of differential thermometry of biologically active points to assess the effectiveness of Meldonium in the treatment of doxorubicin-induced cardiomyopathy.

The choice of cardiomyopathy as an experimental model was determined by the urgency of the problem of detecting and treating this pathology. Indeed, there are currently neither clear clinical, nor functional markers of cardiomyopathy, nor any effective methods of pharmacotherapy or methods of its control have been developed, which significantly complicates the treatment of this disease (Elliott et al. 2008). Besides, patients with this pathology are at high risk of heart failure and death (Maron 2010; O'Mahony et al. 2013; Goroshko et al. 2016).

The doxorubicin cardiomyopathy (CMP) model was chosen as an experimental model for the development of cardiomyopathy. This model reproduces the main metabolic and morphofunctional changes typical of restorative cardiomyopathy (Kolesnichenko et al. 2019).

Cardiomyopathy was modeled in 34 animals by pharmacological means – by four-time administration of doxorubicin (DR), once a week, at a dose of 2 mg/kg (total cumulative dose of 8 mg/kg) intravenously into the marginal vein of the left ear (Lai et al. 2011).

After modeling doxorubicin cardiomyopathy, a morphological study was carried out on 6 animals to assess a degree of toxic myocardial damage. The remaining animals were divided into 2 groups: the 1st group (10 animals) – control (DRCMP) – the animals were administered water for injection at a dose of 2 ml/kg, the 2nd group (10 animals) – Meldonium (DRCMP) – the animals received Meldonium at a dose of 15 mg/kg. The test substances were injected intramuscularly into the back of the thigh, starting from the first day after administering the last dose of doxorubicin, daily for 14 days at the same time of day.

In the second stage of the experiment, the effect of Cortexin in the conditions of the pathological process was assessed by using the model of experimental cerebral ischemia (ECI). Experimental cerebral ischemia was simulated by surgical ligation of the right common carotid artery (Tupikova 1963). The operated animals were divided into two groups: the 1st group –Control (ECI) – was administered water for injection in a volume of 2 ml/kg of body weight; the 2nd group – Cortexin (ECI) at a dose of 0.6 mg/kg. The test substances were injected intramuscularly into

the back of the thigh, starting from the first day after the operation, daily day for 14 days, at the same time of day.

The clinical part of the work was carried out with the participation of 20 patients of both sexes, aged 53 to 83 years diagnosed with an acute cerebrovascula event of the ischemic – stroke type, and 10 healthy volunteers, aged 20–21 years. All the ethical standards were followed throughout the research. The study included patients with ischemic stroke, confirmed in 100% of cases by CT of the brain. Patients with CT-negative stroke, marked brain edema and dislocation with concomitant somatic pathology were excluded from the study.

The studies of the patients were conducted only in the hospital from the 1st to the 14th day of admission to the neurovascular department. All the patients included in the study were divided into 2 groups: Group 1 – SPT, patients received medications included in the standard pharmacotherapy (SPT) to treat ischemic stroke, including antiplatelet agents, anticoagulants, antioxidants, vasoactive drugs, antihypertensive agents, antispasmodics, painkillers, diuretics, vitamins; Group 2 – SPT+CR, patients received Cortexin in a daily dose of 10 mg once intramuscularly during standard pharmacotherapy.

Rationale for choosing the test drugs

Given that the fundamental role in the development of cardiotoxicity when using doxorubicin is connected with activation of lipid peroxidation of membrane structures and disruption of energy exchange, it is natural to use, as a metabolic cardioprotective therapy in patients with doxorubicin cardiomyopathy, substances that block the partial oxidation of free fatty acids and normalize the energy metabolism of cells exposed to hypoxia or ischemia. In particular, at the present stage, Meldonium is of great interest among metabolic drugs (Danilenko et al. 2016; Ezhov et al. 2018).

Meldonium – 3-(2,2,2-Trimethylhydrazinium) propionate – is the most potent reversible inhibitor of gamma-butyrobetainhydroxylase which promotes the synthesis of carnitine from gamma-butyrobetain, thus reducing the carnitine-dependent transport of fatty acids in the mitochondria of muscle tissue and the intensity of beta-oxidation of free fatty acids. Therefore, the use of Meldonium is justified as a drug for the treatment of doxorubicin cardiomyopathy.

The choice of Cortexin is conditioned by the fact that recently more preference has been given to the development of new principles for protecting the brain from ischemic damage –neuroprotection, which can act as a tool for extending the therapeutic window, thereby increasing the effectiveness of reperfusion therapy and preventing secondary damage to neurons after successful reperfusion, as well as making it possible to limit or reduce a degree of damage to nerve cells in the absence of opportunities to administer reperfusion therapy (Odinak et al. 2012).

Cortexin is a drug with a complex of low-molecular-weight peptides (weighing from 1 to 10 kDa) that has a tissue-specific effect on the cerebral cortex. Its pharmacological effect is expressed in neuroprotective, neurotrophic, antioxidant and anticonvulsant actions (Shoboev and Balkhaev 2018). Cortexin suppresses apoptosis and necrosis of nerve cells, restores the viability of neurons, stimulating their growth, and counteracts the ischemic cascade, reducing neurological deficits and raising the general tonus of the central nervous system. The mechanism of action is due to activation of neuronal peptides and neurotrophic factors of the brain, optimization of the level of excitatory and inhibitory mediators, improvement of bioelectric activity of the brain and prevention of free radical formation.

Research methods

To assess the effect of the drugs in the experiment and in the clinic, a continuous recording of the temperature difference between the biologically active point and the intact skin zone was performed, using an autonomous temperature recorder consisting of a differential thermocouple and a digital thermogram registration unit with a serial interface for communication with a personal computer. The Federal Service for Intellectual Property (Russia) issued a utility model patent No. 134028 Device for registering biopotentials and temperatures of biologically active points. The biologically active point was determined using a special probe, basing on topographic and anatomical landmarks. The main thermocouple sensor was placed on the biologically active point, and the second sensor was placed on the intact zone at a distance of 1–1.5 cm.

The dynamics of fluctuations in the temperature differences between the biologically active point and the intact zone of the skin was evaluated continuously for 2 minutes; the changes were recorded every second in the form of thermograms. To describe and assess the dynamics of changes in thermograms, a computer program was proposed and developed that included the calculation of 16 indicators of differential thermometry of biologically active points (Certificate No. 2011611929 dated 02.03.2011). They included:

1. **TNF** – the total number of type I and II fluctuations. An increase in the indicator shows an increase in the intensity of regulatory actions.
2. **TNF/min** – total number of type I and II fluctuations per 1 min. An increase in the indicator shows an increase in the intensity of regulatory actions per 1 min.
3. **NF type I/min** – number of type I fluctuations per 1 min. An increase in the indicator shows an increase in the intensity of type I regulatory actions per 1 min.
4. **NF type II/min** – number of type II fluctuations per 1 min. An increase in the indicator shows an increase in the intensity of type II regulatory actions per 1 min.
5. **R/F F/min** – ratio of the number of type I and II fluctuations per 1 min. The difference between type I and type II regulatory actions in frequency.

6. **DF type I/min** – duration of type I fluctuations in per 1 min. An increase in the indicator shows an increase in the duration of type I regulatory actions per 1 min.
7. **DF type II/min** – duration of type II fluctuations per 1 min. An increase in the indicator shows an increase in the duration of type II regulatory actions per 1 min.
8. **R/F D/min** – ratio of the duration of type I and II fluctuations per 1 min. The difference between type I and type II regulatory actions in duration.
9. **FRI** – frequency regulation index (the quotient of the number of type I and II fluctuations per 1 min). The predominance of the frequency of regulatory actions, if type II < 1 cu. > type I.
10. **DRI** – duration regulation index (the quotient of the duration of type I and II fluctuations per 1 minute). The predominance of the duration of regulatory actions, if type II < 1 cu. > type I.
11. **AVA type I/2 min** – the average value of the amplitude of type I fluctuations per 2 min. An increase in the index shows an increase in the intensity of the range of type I regulatory actions.
12. **AVA type II/2 min** – the average value of the amplitude of type II fluctuations per 2 min. An increase in the index shows an increase in the intensity of the range of type II regulatory actions.
13. **FAF/min** – frequency of absence of fluctuations per 1 min. Absence of heterogeneity of regulatory actions in frequency. An increase in the index indicates a decrease in the intensity of regulatory actions.
14. **DAF/min** – duration of absence of fluctuations per 1 min. Absence of heterogeneity of regulatory actions in duration. An increase in the index indicates a decrease in the intensity of regulatory actions.
15. **H. Coef.** – heterogeneity coefficient. A decrease in heterogeneity coefficient indicates a shift in the established relationship between regulatory actions and their absence on the thermogram within 2 minutes towards a certain decrease in regulatory processes, whereas an increase in H. Coef., on the contrary, indicates a shift towards their increase.
16. **% DF/2min** – percentage of the duration of fluctuations per 120 seconds. The indicator gives an idea of a share of regulatory actions that occurred for 2 minutes.

Type I fluctuation is a periodic change in the temperature difference from the isoline (stationary temperature difference) towards an increase in the temperature of the BAP; type II, on the contrary, – towards its decrease.

Using these parameters, it is possible to assess the process of forming regulatory actions in real-time, thus getting an idea of the effect of drugs.

The values of the indicators were expressed in counting units (cu).

In the experimental part of the study, the temperature difference was registered in all the rabbits before the pathology simulation (of doxorubicin cardiomyopathy and of experimental brain ischemia) on the 1st day (the beginning of treatment), on the 7th and 14th day of injecting the medicines.

The temperature difference was registered in all the rabbits on the right ear. The biologically active point of the heart meridian was determined using a special probe and basing on topographic and anatomical landmarks (Portnov 1987).

In the clinical part of the study, the examination was performed by continuously registering the temperature difference between biologically active point C7 and the intact skin zone 3 times: 1st – on the 1st day, for patients it was the day of admission to hospital, 2nd – on the 7th day, 3rd – on the 14th. Biologically active point C7 of the heart meridian was determined using a special probe and basing on topographic and anatomical landmarks (Tabeeva 2016).

Simultaneously with recording the temperature difference between the biologically active point and the intact skin zone, all the rabbits with doxorubicin cardiomyopathy were examined for blood biochemical parameters (alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine phosphokinase (CPK)) and given an electrocardiogram (ECG). The ECG was assessed by the following parameters: heart rate, duration of the PQ interval, and the presence and nature of the rhythm disturbances and repolarization. There were 3 degrees of ECG changes identified: I – reduced R and/or T wave voltage , II – T-wave inversion, III – ST-segment elevation and/or the appearance of pathological Q-wave (Stolyarova 2004).

A morphological study was performed on all the animals on the 14th day. For review purposes and visualization of morphological changes in cardiomyocytes, cortex of the frontal lobe of the large hemispheres and hippocampus, the obtained sections were stained with hematoxylin and eosin. To identify the damaged areas of the myocardium with ischemic and metabolic disorders, the sections were stained using the Lie staining (hematoxylin, basic fuchsin, picric acid) (Atyakshin and Bukhvalov 2016). For a general histological analysis of a degree of damage to brain tissues, the obtained sections were stained with toluidine blue (Nissl staining) and with hematoxylin-eosin.

Morphometry was performed on the images obtained in 10 fields of view at 100× magnification. Morphometry measured: 1) the thickness of cardiomyocytes – the maximum cross-section size of the cardiomyocyte, mcm; 2) the diameter of the capillary lumen – a gap between the endothelial cells of the capillary in cross-section, mcm. The ratio of the capillary diameter to the cardiomyocyte thickness was also determined – this coefficient allows assessing the dynamics of morphological changes in the myocardium.

The metabolic state of the myocardial contractile elements was assessed by counting fuchsinophilic cardiomyocytes in the field of view using the visualization complex with the Micro-Analysis View software package with the X40 lens (LOMO-Microsystems Join Stock Company, Russia). The tested areas of the micro-product did not

intersect with one another. To get an objective view of the level of metabolic disorders of the myocardium, at least 100 visual fields were analyzed in each section.

The digital data were processed using Microsoft Excel 2013.

For an objective assessment of the severity of the ischemic stroke and control of treatment, a comprehensive assessment was used according to the clinical and neurological scales: NIHSS (Sumin et al. 2016), Original (Mitrokhin et al. 2014), WAM (well-being, activity, mood) scale (Molchanova and Grishchenko 2017), and Bartel scale (for assessing a social adaptation level) (Sumin et al. 2016).

Statistical data processing

When the data were mathematically processed, the arithmetic mean (M) and the standard error of the arithmetic mean (±m) were calculated. The significance of differences between the groups was calculated using parametric (more than 16 objects of the Student's t-test) and non-parametric estimation (less than 16 objects according to the Wilcoxon rank criterion). The data with a significance level of $p < 0.05$ were considered significant. To study the relationship between two random variables of two data sets, a correlation analysis was performed. This analysis was carried out in two directions: 1) canonical analysis; 2) correlation analysis.

Within the framework of the canonical analysis, the multidimensional closeness of the relationship between the elements of the two sets was estimated. Combinations of values with the maximum correlation were found in each set. The resulting canonical roots explain the unique share of variability between the two sets of variables. The number of canonical roots corresponds to the dimension of the smallest of the two sets. In the experiment, the 1st set was represented by the data obtained through the standard research methods (biochemical blood test and ECG); the 2nd set was represented by 16 indicators of differential thermometry of BAP. In the clinic, the 1st set was represented by the data obtained from clinical and neurological scales, and the 2nd set was represented by 16 indicators of differential thermometry of BAP.

To study the relationship by determining a degree of probability of a connection between two or more random variables (between each indicator of the temperature difference and each scale separately), a correlation analysis was used, with calculating the correlation coefficient (rs). Reliable estimates of correlation coefficients were classified according to the following scale:

- rs < 0.3 – weak correlation,
- 0.3 rs < 0.70 – moderate correlation,
- rs > 0.70 – strong correlation.

Statistical processing of the obtained material was performed using the statistical analysis packages Microsoft Excel 2007 and PASW (ex.SPSS) V.17.0.2. (demo version) in OS Windows 7.

Results and discussions

Experimental part. Stage 1

The development of cardio- and cyto-toxic effects of doxorubicin after its administration at a total cumulative dose of 8 mg/kg was confirmed by standard research methods. When assessing ECG in all the animals, an increase in the heart rate was recorded by 14% ($p < 0.05$). Also, when assessing the nature of rhythm disturbances and repolarization, 54% of animals showed changes characteristic of degree III myocardial damage, 37% – degree II, and 9% – degree I. The administration of doxorubicin led to a significant increase in blood biochemical parameters, such as ALT, AST, CPK, and LDH. The observed morphofunctional changes in cardiomyocytes indicated a different degree of severity of non-coronarogenic dystrophic and necrotic changes.

When conducting differential thermometry of BAP, there was a decrease in the 1st, 2nd, 3rd, 4th, 6th and 7th indicators by 22% or more, as well as an increase in the 14th indicator by 20% or more.

These data indicate that the administration of doxorubicin at a total cumulative dose of 8 mg/kg leads to a decrease in the intensity of regulatory processes, which, most likely, can be considered a consequence of the development of its cardio- and cyto-toxic effects.

The use of Meldonium for 14 days made it possible to limit myocardial damage and the development of tachycardia, as well as to limit an increase in blood biochemical parameters (ALT, AST, CPK, and LDH). Therefore, there is a positive therapeutic effect of Meldonium when used in the conditions of doxorubicin myocardial damage.

When Meldonium was administered on day 7, the heart rate still exceeded the baseline by 15% ($p < 0.05$), decreased on day 14 and did not differ significantly from the baseline, while in the control group, when water was injected, the heart rate exceeded the baseline for 14 days (Table 1). In the animals treated with Meldonium, ECG signs of degree I myocardial damage prevailed on both the 7th and 14th day, whereas no degree III myocardial damage was observed. In the control group, III degree damage remained predominant on the 7th day, amounting to 44.5% of all the cases, and on the 14th day it was observed in 1/3 of the animals (Fig. 1).

On the 7th day of administrating Meldonium to the animals with doxorubicin cardiomyopathy, ALT and AST levels decreased to the same extent as in the control group

Table 1. Heart Rate in the Simulated Doxorubicin Cardiomyopathy in Rabbits and Throughout Its Treatment (M ± m).

Test groups	Initial values	Doxorubicin cardiomyopathy – 1st day	7th day	14th day
Control group (DRCMP)	285 ± 15*	289 ± 8.5*	275 ± 12*	285 ± 15*
Meldonium group (DRCMP)	235.7 ± 8.3	277.5 ± 11*	272.1 ± 13.7*	245 ± 8.3#

Note: * – $p < 0.05$ – differences are significant compared to the baseline level; # – $p < 0.05$ – differences are significant compared to the 1st-day administration of the last dose of doxorubicin.

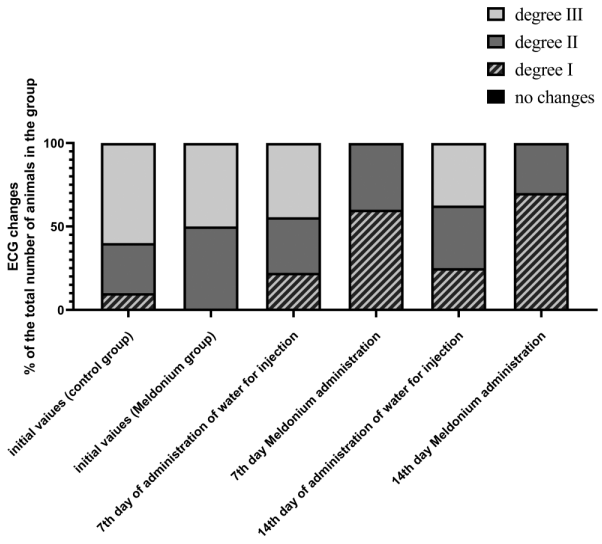

Figure 1. ECG changes in the simulated doxorubicin cardiomyopathy in rabbits and throughout its treatment (% of the total number of animals in the group).

and did not differ significantly from the initial values. LDH and CPK remained at a significantly high level in blood plasma, exceeding the level of the healthy animals by 200% and 196%, respectively, but they were significantly lower than the level of the control group. Further administration of Meldonium led to a decrease in the level of the studied blood biochemical parameters to the levels registered before the experiment, as well as in the control group (Table 2). As there were no significant differences in the dynamics of changes in blood biochemical parameters in the control (DRCMP) and experimental (DRCMP) groups, it can be concluded that their control provides little information for assessing the effectiveness of Meldonium in the treatment of doxorubicin cardiomyopathy.

The morphological study of the left ventricular myocardium of the animals treated with Meldonium in comparison with the animals of the control group (DRCMP) revealed a significant increase in myocardial hypertrophy, reaching the maximum values.

The number of fucsinophilic cardiomyocytes obtained by the Lie staining in the field of view of the animals of the Meldonium group (DRCMP) was 3.6 times higher than the number obtained in the intact animals and 2.3 times lower than in the group of the animals with doxorubicin cardiomyopathy (Table 3, Fig. 2), which indicates a decrease in the severity of metabolic and ischemic myocardial damage when Meldonium was administered.

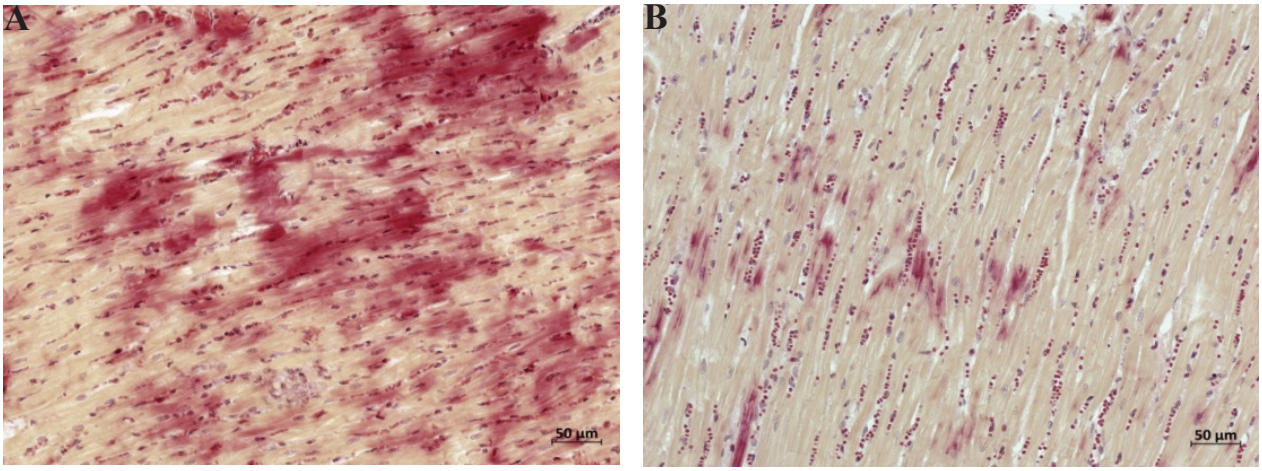

Figure 2. Morphological picture of the left ventricular myocardium. **A** – when administering water for injection to animals with doxorubicin cardiomyopathy for 14 days (a moderate number of diffusely located medium foci of moderate fuchsinophilia of the cardiomyocyte cytoplasm); **B** – when administering Meldonium to animals with doxorubicin cardiomyopathy for 14 days (a small number of diffusely located medium foci of weak and moderate fuchsinophilia of the cardiomyocyte cytoplasm). Staining: Lie staining method, 20×.

Table 2. Dynamics of Blood Biochemical Parameters When Injecting Water and Meldonium to Animals With Doxorubicin Cardiomyopathy (M ± m).

Biochemical blood values	Initial values	On the 1st day after injecting the last dose of doxorubicin	7th day of drug administration	14th day of drug administration
Control group (DRCMP)				
ALT	41.57 ± 7.21	76.57 ± 6.24*	56 ± 9.39#	52.57 ± 6.62#
AST	25.14 ± 3.15	68.57 ± 5.35*	45.57 ± 13.43*#	31.29 ± 3.39#
LDH	209.86 ± 24.11	578.14 ± 24.69*	586.29 ± 48.44*	304.86 ± 48.44#
CPK	283.71 ± 18.82	1055.143 ± 24.9*	866.57 ± 69.06*	368.86 ± 19.47#
Meldonium group (DRCMP)				
ALT	40.71 ± 3.03	75.86 ± 7.08*	55.14 ± 7.03#	37.57 ± 3.67#
AST	26.14 ± 3.15	69 ± 6.35*	36.71 ± 6.93#	27.71 ± 4.93#
LDH	197.86 ± 3.93	577 ± 28*	395.43 ± 26.58*#	268.14 ± 27.51#
CPK	284.71 ± 12.02	1027 ± 11.04*	557.71 ± 37.93*#	335.86 ± 28.94#

Note: * – $p < 0.05$ – compared to the values obtained before drug administration; # – $p < 0.05$ – the differences are significant compared to the 1st day of administration of the last dose of doxorubicin.

Table 3. Number of Cardiomyocytes With Signs of Ischemic Disorders (on the Visual Field, Lie Staining Method) (M ± m).

Groups	Number of cardiomyocytes with the signs of cytoplasmic fuchsinophilia
Intact animals	1.9 ± 0.1
Animals with simulated doxorubicin cardiomyopathy	15.7 ± 1.3*
Meldonium (DRCMP)	6.9 ± 0.6*#
Control group (DRCMP)	14.6 ± 0.9*

Note: * – significant in relation to healthy animals; # – significant in relation to animals with cardiomyopathy.

Therefore, the use of Meldonium as a means of therapy for doxorubicin cardiomyopathy leads to significant favorable changes in the heart tissue, strengthening compensatory-adaptive and reparative processes.

In the study of the parameters of differential thermometry of BAP in response to the introduction of Meldonium on the 7th day, there was observed an increase in the number and duration of fluctuations of the temperature difference and a decrease in the time periods with their absence on the thermograms compared to the start of treatment, which indicated an increase in the intensity of regulatory actions monitored by the following indicators: TNF, TNF/min, NF type I/min, NF type II/min, DF type I/min, DF type II/min, H. Coef, %DF/2min, and DAF/min (Figs 3, 4). At the same time, the most pronounced increase was observed on the 7th day of treatment in terms of TNF (1), TNF/min (2), NF type I/min (3), DF type II/min (7) and % DF/2 min (16), which amounted to more than 60%.

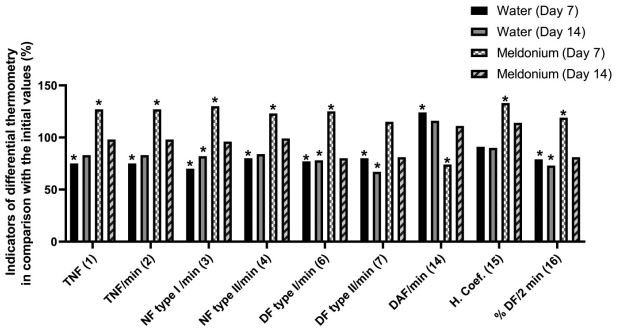

Figure 3. Dynamics (%) of indicators of differential thermometry of BAP on the 7th day of injection of water and Meldonium to animals with doxorubicin cardiomyopathy compared to the initial values. **Note:** * – $p < 0.05$.

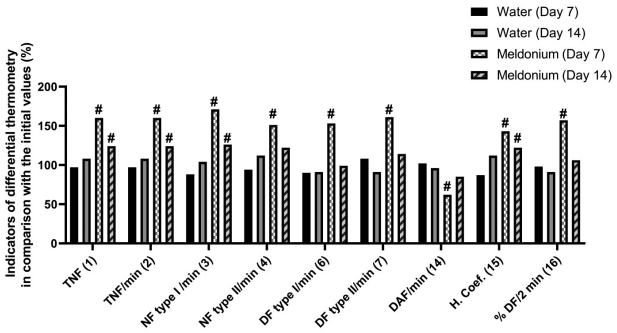

Figure 4. Dynamics (%) of indicators of differential thermometry of BAP on the 7th and 14th days of injecting water and Meldonium to animals with doxorubicin cardiomyopathy compared to the values obtained on the 1st day after administering the last dose of doxorubicin. **Note:** * – $p < 0.05$.

On the 14th day of administration of Meldonium, the intensity of regulatory processes estimated by the indicators of differential thermometry of BAP was stabilized and did not differ from the initial level of the healthy animals.

In the control experiment with administering water for injection, there were no changes of the indices of differential thermometry of BAP compared to the beginning of doxorubicin cardiomyopathy treatment on the 7th and 14th days – the parameters of BAP differential thermometry remained significantly below the initial level, which corresponded to the healthy animals. The 1st, 2nd, 4th, 7th, 15th, and 16th indicators declined by 20% or more. The exception was the 14th indicator, which, on the contrary, increased, indicating the absence of heterogeneity of regulatory actions by duration (Figs 3, 4).

The final task of this stage of the experiment was an analysis of a multidimensional correlation between two sets: the 1st set is represented by the data obtained by standard methods of research (biochemical blood analysis and ECG), the 2nd set is represented by 16 indicators of differential thermometry of BAP. The resulting canonical values of R are quite large and highly significant ($p < 0.001$) in both groups, which confirms a significant correlation between the biochemical parameters of blood, ECG and indicators of differential thermometry BAP (Table 4).

Therefore, the data analysis makes it possible to conclude that generally the information obtained from the biochemical analysis of blood and ECG is duplicated by information on 16 indicators of differential thermometry of BAP.

Analyzing the dynamics of changes in ECG and blood biochemical parameters, as well as morphological changes happening in the myocardial tissues during the development of doxorubicin-induced cardiomyopathy against the administration of water for injection and Meldonium, it should be noted that they are parallel with the dynamics of changes in the indicators of differential thermometry of BAP. This, together with the data on the significant correlation of methods, makes it possible to state that the method of differential thermometry of BAP in real-time provides an opportunity to assess the effectiveness of pharmacotherapy and personalize the treatment. In particular, the course administration of Meldonium at a dose of 15 mg/kg for 14 days has a protective effect in the treatment of doxorubicin cardiomyopathy which is expressed in limiting myocardial damage, the development of tachycardia, and reducing blood biochemical parameters (ALT, AST, CPK, and LDH), as well as in increasing the intensity of

Table 4. Overall Results of the Canonical Analysis of Control (DRCMP) and Meldonium (DRCMP) Groups.

Indicators	Control group (DRCMP)		Meldonium (DRCMP)	
	16 indicators	6 indicators	16 indicators	6 indicators
Canonical value Rc	0.853*		0.940*	
Number of variables	14	6	16	6
Extracted dispersion, %	44.271	100.000	56.456	100.000
Total redundancy, %	18.221	38.488	18.782	57.678

Note: * – $p < 0.01$.

regulatory processes, estimated by the indicators of TNF, TNF/min, NF type I/min, DF type II/min and %DF/2min by 60% or more on the 7th day of treatment with the above parameters approaching the norm on the 14th day.

Experimental part. Second stage

At the second stage, the effect of Cortexin was assessed in the conditions of pathological process – experimental brain ischemia caused by ligation of the right common carotid artery. It turned out that 24 hours after ligation of the right common carotid artery, there was an increase in most indicators of differential thermometry of BAP (1st, 2nd, 3rd, 4th, 6th, 7th, and 15th) by 18% or more, as well as a decrease in the 13th and 14th by 11% or more (Table 5).

Table 5. Some Indicators of Differential Thermometry of BAP in Healthy Rabbits and Experimental Brain Ischemia for 1 Day (cu, M ± m, n < 55).

Indicators of differential thermometry of BAP	Registration time		% change relative to the initial level
	Initial level – before operation	1st day after operation	
1	41.65 ± 0.79	49.47 ± 0.84*	118.8
2	20.95 ± 0.41	24.73 ± 0.45*	118
3	10.38 ± 0.25	12.24 ± 0.28*	117.9
4	10.58 ± 0.23	12.49 ± 0.26*	118
6	11.82 ± 0.27	14.01 ± 0.29*	118.5
7	11.57 ± 0.25	13.85 ± 0.29*	119.7
13	11.12 ± 0.26	9.87 ± 0.26*	88.8
14	35.71 ± 0.65	30.71 ± 0.78*	86
15	1.88 ± 0.05	2.52 ± 0.08*	133.7
16 (%)	39 ± 0.78	47 ± 0.88*	120.5

Note: * – p < 0.05 – differences are significant compared to the initial level; cu – calculation units.

Therefore, these indicators reflect the activation of regulatory processes, which must be a response to an acute pathological process – brain ischemia.

The use of Cortexin in experimental brain ischemia on the 7th day caused a further even more pronounced activation of regulatory processes estimated by the indicators of differential thermometry of BAP. Thus, indicators 2, 4, 6, 7 and 16 increased by 15% or more compared to the beginning of the treatment, and by 30% or more compared to the initial values (Table 6).

Later on the 14th day in the Cortexin group (ECI), the indicators of differential thermometry of BAP reached the initial values and did not differ significantly from them (Table 6).

In the control (ECI) group of the animals injected with water, on the contrary, there was a negative dynamics, which was expressed in a decrease in the indicators of differential thermometry of BAP on the 7th day and the 14th day compared to the initial level, taken as a conditional norm, and compared to the 1st day after the operation (Table 7), which indicated a decrease in the regulation processes.

The morphological study of the frontal cortex of the large hemispheres and the hippocampus revealed that the most significant morphofunctional changes in neurons that developed during experimental ischemia were detected in the group of animals that had been injected with water (Fig. 5). Minimal damage to the brain tissue after ischemia was detected when using Cortexin (Fig. 6).

Analyzing the dynamics of changes in the morphological picture occurring in the brain tissues during its ische-

Table 6. Some Indicators of Differential Thermometry of BAP When Aministering Cortexin to Animals With Experimental Brain Ischemia (cu, M ± m, n < 11).

Indicators of differential thermometry of BAP	Registration time			
	Initial level – before operation	1st day after operation	7th day	14th day
1	39.73 ± 1.27	46.60 ± 1.48*	51.91 ± 1.52*	38.91 ± 1.61 #
2	19.55 ± 0.71	22.80 ± 0.74*	25.86 ± 0.85*#	19.45 ± 0.80#
3	10.01 ± 0.48	11.43 ± 0.40	12.73 ± 0.53*	10.12 ± 0.49
4	9.59 ± 0.37	11.36 ± 0.48*	13.10 ± 0.55*#	9.15 ± 0.44 #
6	11.94 ± 0.51	14.02 ± 0.60*	16.55 ± 0.59*#	11.83 ± 0.51#
7	11.17 ± 0.54	13.30 ± 0.52*	15.51 ± 0.60*#	10.94 ± 0.52 #
14	34.98 ± 1.24	28.33 ± 1.05*	23.63 ± 1.26*#	37.17 ± 1.30#
15	1.85 ± 0.08	2.26 ± 0.13*	2.57 ± 0.19*	1.98 ± 0.16
16 (%)	38 ± 1.57	45 ± 1.78*	53 ± 1.93*#	38 ± 1.58#

Note: * – p < 0.05 – differences are significant compared to the initial level; # – p < 0.05 compared to the values obtained on the 1st day after ligation of the right common carotid artery; cu – calculation units.

Table 7. Some Indicators of Differential Thermometry of BAP When Injecting Water to Animals With Experimental Brain Ischemia (cu, M ± m, n < 11).

Indicators of differential thermometry of BAP	Registration time			
	Initial level – before operation	1st day after operation	7th day	14th day
1	38.18 ± 1.58	45.55 ± 1.45*	36.00 ± 1.43 #	30.50 ± 1.54*#
2	19.53 ± 0.70	22.85 ± 0.75*	17.85 ± 0.93 #	14.97 ± 0.82*#
3	9.19 ± 0.43	10.69 ± 0.48*	8.53 ± 0.42 #	7.76 ± 0.57#
4	10.34 ± 0.45	12.16 ± 0.51*	9.32 ± 0.61 #	7.12 ± 0.47*#
6	10.51 ± 0.38	12.81 ± 0.46*	9.56 ± 0.51 #	8.03 ± 0.43*#
7	11.75 ± 0.51	14.13 ± 0.53*	9.63 ± 0.46*#	7.38 ± 0.41*#
14	37.13 ± 1.22	34.41 ± 1.29	39.52 ± 1.29#	42.72 ± 1.33*#
15	1.67 ± 0.07	2.64 ± 0.20*	2.09 ± 0.14*#	2.43 ± 0.24*
16 (%)	37 ± 1.29	45 ± 1.44*	32 ± 1.39*#	26 ± 1.35*#

Note: * – p < 0.05 – differences are significant compared to the initial level; # – p < 0.05 compared to the values obtained on the 1st day after ligation of the right common carotid artery; cu – calculation units.

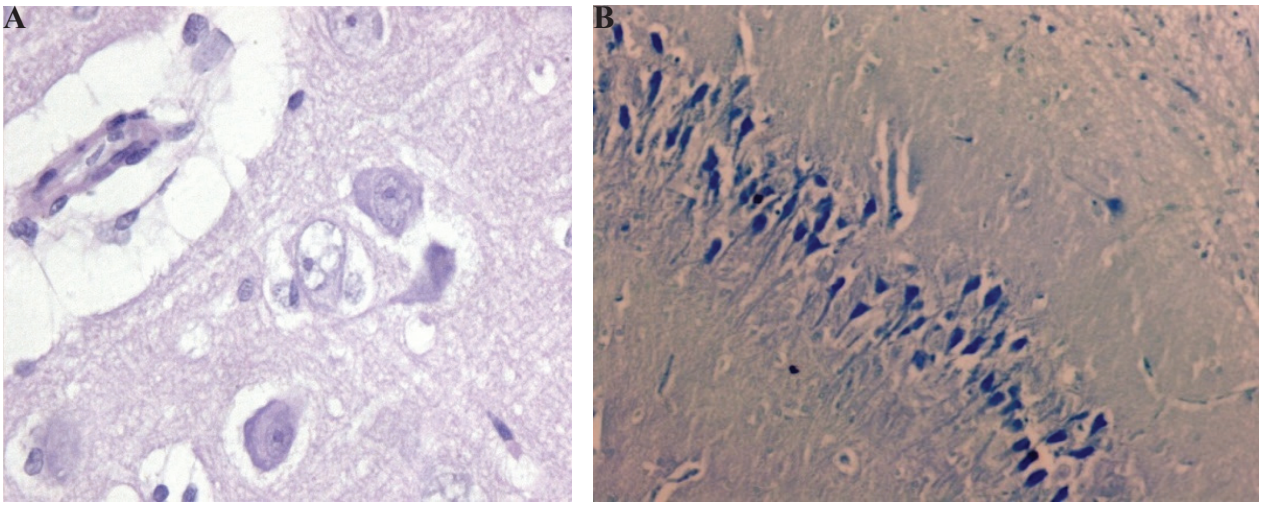

Figure 5. The state of the frontal cortex of the major hemispheres (**A**) and hippocampus (**B**) when injecting water to animals with experimental brain ischemia for 14 days. Nissl staining. A – vacuolation of the cytoplasm and nucleus of neurocytes, pronounced perivascular edema; 630×. A decrease in the density of neurons in the frontal cortex of the large hemispheres of the brain; 100×. B – hyperchromia and pyknosis of hippocampal neurocytes; 100×. G – pericellular edema; 630×.

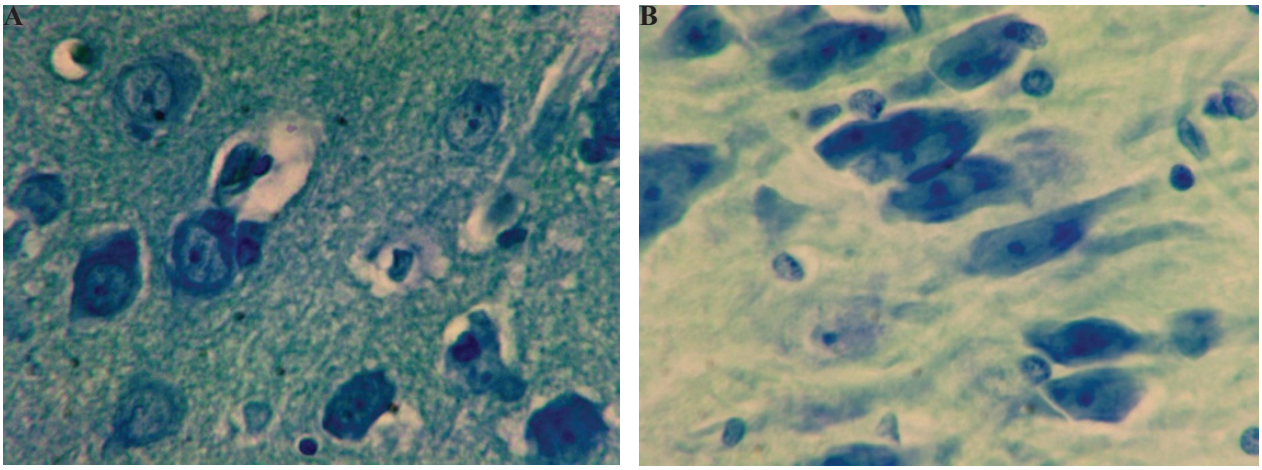

Figure 6. Morphological picture of the frontal cortex of the large hemispheres (**A**) and hippocampus (**B**) when Cortexin was administered to animals with experimental brain ischemia for 14 days. Nissl staining. Actively functioning hyperchromic nerve cells without signs of alterations. 400×.

mia when administrating Cortexin, it should be noted that they were parallel with the dynamics of changes in the indicators of differential thermometry of BAP. The best recovery phenomena in the central nervous system were an increase in the intensity of regulatory actions within the 1st and 2nd minutes (TNF and TNF/min), the frequency and duration of regulatory actions of both types I and II (NF type I/min, NF type II/min, DF type I/min and DF type II/min), the coefficient of heterogeneity (H. Coef.) and the percentage of regulatory processes per 2 min (%DF/2min), as well as a reduction in the duration of the absence of heterogeneity of regulatory processes (DAF/min) on the 7th day of drug administration with the above parameters reaching the norm on the 14th day. Insignificant recovery events in the central nervous system, on the contrary, were a decrease in the above criteria on the 7th and further on the 14th day of treatment.

Clinical part

The clinical study was conducted to assess in more detail the effectiveness of treatment of patients with ischemic stroke with a standard set of medications, as well as when Cortexin was included in the treatment program. Before conducting the clinical testing, during the study period (14 days) it was established in healthy individuals that there were no significant differences in the studied indicators of differential thermometry for BAP C7 (Fig. 7A, B).

When assessing the treatment of the patients with ischemic stroke on the neurological scales of ischemic stroke severity 14 days later, positive dynamics was noted in all the test groups. Higher effectiveness compared to that in the control group was shown by a study conducted in the group of patients who had been prescribed Cortexin in combination with the standard pharmacotherapy. In that

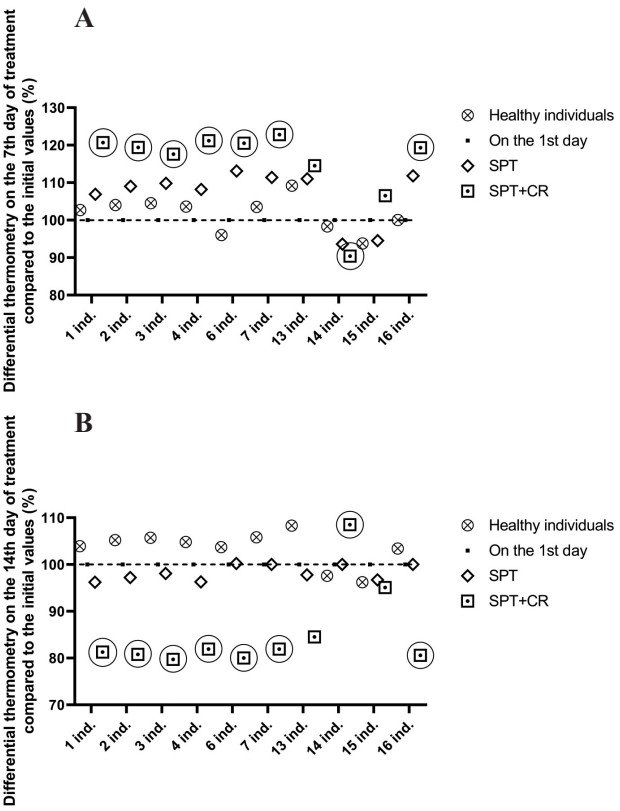

Figure 7. The main indicators of differential thermometry of bap c7 (%) on the 7th day (**A**) and on the 14th day **(B**) of treatment in patients of spt and spt+cr groups compared to the values obtained at admission. **Note:** on the ordinate axis – %, on the abscissus axis – indicators of differential thermometry; – $p < 0.05$. Compared to day 1.

group, regression of neurological symptoms was the most pronounced. The use of Cortexin contributed to both an increase in the quality of life index, the self-care abilities and a reduced need for care, and to the most optimistic assessment of their psycho-emotional level, which can confirm the high rehabilitation potential of the drug (Table 8).

During the analysis of indicators of differential thermometry of BAP C7, it was found that in the treatment of patients with ischemic stroke only with a standard set of medications, there were no significant differences in their values on day 7. When prescribing Cortexin, there was an increase in the indicators. When administering Cortexin, a significant increase was recorded for 7 indicators: TNF (1), TNF/min (2), NF type I/min (3), NF type II/min (4), DF type I/min (6), DF type II/min (7), and %DF/2min (16). These data indicate that there is an increase in regulatory processes by the 7th day when Cortexin was introduced into the treatment program. The duration of the absence of heterogeneity of regulatory actions decreased (DAF/ min (14)) (Fig. 7A).

Further treatment with administering Cortexin on the 14th day led to a decrease in the intensity of regulatory processes below the level obtained at admission to hospital. There was also an increase in the duration of the absence of heterogeneity of regulatory processes (DAF/ min (14)). Similarly to the 7th day in the group of patients who had received a standard set of medicines, there were no significant differences in the activity of regulatory processes (Fig. 7B).

The study also looked at how the indicators of differential thermometry of BAP correlated with the changes that characterize the neurological status, which was implemented in two ways: by means of the canonical analysis that evaluated the multidimensional closeness of the relationship between the elements of the two sets – the 1st, represented by 6 neurological scales, and the 2nd, represented by 16 indicators of differential thermometry and a correlation analysis. The number of canonical roots corresponds to the dimension of the smallest of the two sets, in the present study there were 6 of them. This statistical method is particularly useful in research since two large sets of variables are available, and of interest for the present study was a degree of relationship between them during the treatment.

Table 9 shows the results of checking the significance of the extracted canonical roots. The study used the following sequential procedure for checking the significance. First, all six canonical variables are considered without removing the roots. The resulting value has a high statistical significance, as evidenced by the low value of the type-one error ($p < 0.01$). Then, starting from the first one, the roots are "discarded", and the statistical significance of the remaining roots is determined. Since in the case in question all six roots in both test groups were significant, it can be concluded that the measurements on six neurological scales correspond to the measurements on sixteen indicators of differential thermometry of BAP C7.

Table 8. Changes in the Indicators of Neurological Status, Psychoemotional Status and Level of Social Adaptation and Quality of Life According to Clinical and Neurological Scales in Patients of SPT and SPT+CR groups (M ± m, n < 10).

Scales	Groups			
	Standard pharmacotherapy		Standard pharmacotherapy + Cortexin	
	Before treatment	After treatment	Before treatment	After treatment
NiHSS	11.70 ± 0.73	9.40 ± 0.88*	11.30 ± 0.58	6.80 ± 0.70*
Original	36.50 ± 1.09	38.60 ± 1.23	37.20 ± 1.13	42.50 ± 1.35*
Well-being	2.09 ± 0.14	3.45 ± 0.26*	2.09 ± 0.12	4.15 ± 0.29*
Activity	2.26 ± 0.10	3.52 ± 0.24*	2.24 ± 0.13	4.22 ± 0.25*
Mood	2.01 ± 0.11	3.45 ± 0.29*	2.23 ± 0.18	4.29 ± 0.31*
Bartel	48.50 ± 3.58	59.50 ± 3.20*	47.78 ± 3.27	72.5 ± 3.27*

Note: * – $p < 0.05$ – differences are significant compared to values obtained before treatment.

Table 9. Significance Check of the Extracted Canonical Roots of the SPT and SPT+CR groups.

Number of discarded roots	BAP C7, SPT group		BAP C7, SPT+CR group	
	Canonical R_c	Probability of a type-one error (p-level)	Canonical R_c	Probability of a type-one error (p-level)
0	0.999*	0.000	0.999*	0.000
1	0.977*	0.000	0.981*	0.000
2	0.960*	0.000	0.948*	0.000
3	0.851*	0.000	0.833*	0.000
4	0.771*	0.000	0.734*	0.000
5	0.757*	0.000	0.592*	0.015

Note: * – $p < 0.01$.

The analysis of standardized values of canonical weights (Table 10) shows a unique contribution of the original variables to the formation of canonical variables. The greatest contribution to the formation of the first canonical variable of the left set is made by original variable 2, represented by the Original clinical and neurological scale.

Table 10. Canonical Weights of the Left Set Represented by the Results of Clinical and Neurological Scales (SPT+CR groups).

No. of variable	Root 1	Root 2	Root 3	Root 4	Root 5	Root 6
1	-0.783	-0.279	1.776	0.511	0.263	0.195
2	-1.213	0.043	-0.105	0.048	-0.260	-0.153
3	-0.704	-5.698	0.932	-3.461	-3.309	-0.081
4	0.337	5.480	1.847	0.498	0.207	2.144
5	0.345	0.765	-1.835	2.195	3.293	-2.771
6	0.244	-0.642	0.934	1.442	-0.398	0.159

The canonical weights were also calculated for the right set (Table 11). The greatest influence on the formation of the first canonical variable in the right set was exerted by original variables 2, 3, 4, 6, and 7. In other canonical variables, the influence of variables 2, 3, and 4 is clearly expressed. Consequently, the most closely correlated indicators are TNF/min (2), NF type I/min (3), NF type II/min (4), DF type I/min (6) and DF type II/min (7).

Table 11. Canonical Weights of the Right Set Represented by the Indicators of Differential Thermometry of BAP (SPT+CR Groups).

No. of variable	Root 1	Root 2	Root 3	Root 4	Root 5	Root 6
1	0.231	-0.768	-0.850	0.586	0.350	0.960
2	-5.446	-151.111	87.524	-73.065	-103.304	157.117
3	4.789	75.876	-43.630	22.965	48.781	-78.935
4	4.167	76.512	-46.840	58.709	65.359	-74.151
5	0.235	-1.747	0.127	3.355	1.936	0.910
6	-2.302	7.781	-2.365	2.895	1.655	-15.547
7	-2.443	-6.373	7.565	-11.739	-12.989	8.055
8	-0.393	-1.814	1.568	-1.083	-4.040	2.849
9	-0.105	0.904	-0.615	0.004	0.087	0.501
10	-0.052	0.278	0.479	-0.100	0.720	-0.072
11	1.061	-0.112	-0.024	1.007	2.863	-0.263
12	-0.438	-0.472	0.429	-1.308	0.773	-0.601
13	-0.002	-0.650	-0.122	0.721	-1.055	0.006
14	-1.032	1.238	1.839	-0.586	-1.691	-2.502
15	-0.936	0.037	0.891	-0.111	-0.713	-0.901
16	-2.412	3.025	4.184	-0.661	-2.655	-4.197

Thus, the data analysis makes it possible to conclude that generally the measurements on the six clinical and neurological scales are duplicated by the measurements on the sixteen indicators of differential thermometry.

The relationship of each indicator of differential thermometry of BAP with neurological scales individually was traced in the analysis of correlation coefficients. The analysis of the relations between the indicators of differential thermometry of BAP and the neurological scales revealed a moderate correlation (0.3> rs <0.70) and a strong correlation (rs>0.70) between some combinations on the 14th day. At the same time, there was no correlation between other combinations. However, these data do not make it possible to exclude the relationship between the two presented methods.

For example, the closest relationship was observed with the Original scale, as 12 out of 16 indicators correlated with it, and the "activity" component of the WAM scale – 12 out of 14. It is also worth noting that there was a negative correlation relationship with the Bartel scale by 11 indicators of differential thermometry (TNF (1), TNF/min (2), NF type I/min (3), NF type II/min (4), R/F F/min (5), DF type I/min (6), DF type II/min (7), R/F D/min (8), FAF/min (13), H. Coef. (15), and %DF/2min (16), while the correlation coefficient was below -0.6.

The above materials are enough to establish more informative indicators in the differential thermometry of BAP that characterize the effect of drugs in the experimental and clinical conditions. These indicators are: the 1st (TNF), 2nd (TNF/min), 3rd (NF type I/min), 4th (NF type II/min), 6th (DF type I/min), 7th (and DF type II/min), 14th (DAF/min), 15th (H. Coef.), and 16th (%DF/2 min) indicators. The fact that there was a correlation identified between changes in the temperature difference and the dynamics of neurological status, psychoemotional state and the level of quality of life of patients, made it possible to interpret the dynamics of indicators of differential thermometry of BAP on the 7th and 14th days. Since when injecting Cortexin, the dynamics of recovery of neurological impairments on clinical neurological scales were more significant, it can be assumed that an increase in the intensity of regulation processes in BAP C7 on the 7th day of treatment by more than 20% in terms of TNF (1), NF type II/min (4), DF type I/min (6), DF type II/min (7) and a decrease in them by the 14th day confirms the favorable dynamics of recovery of the disturbed functions and the effectiveness of pharmacotherapy.

Conclusion

This clinical and experimental work resulted in the development of a safe and non-invasive method for monitoring the effects of drugs in real-time, which does not requiry any special training of a doctor and which is based on the continuous registration of changes in the body's regulatory systems described quantitatively using indicators of differential thermometry of BAP. A comparison of the degree of differences between the thermogram indicators before the drug is administered and during its action (at certain time intervals) allows assessing the effect of a pharmacological or other means on the animal and human body. Therefore, it is recommended to use the method of differential thermometry of biologically active points to assess the effect of drugs on the 7th and 14th days of therapy in patients with brain and heart damage to optimize the treatment.

Conflict of interest

The authors declare no conflict of interest.

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Author Contributions

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