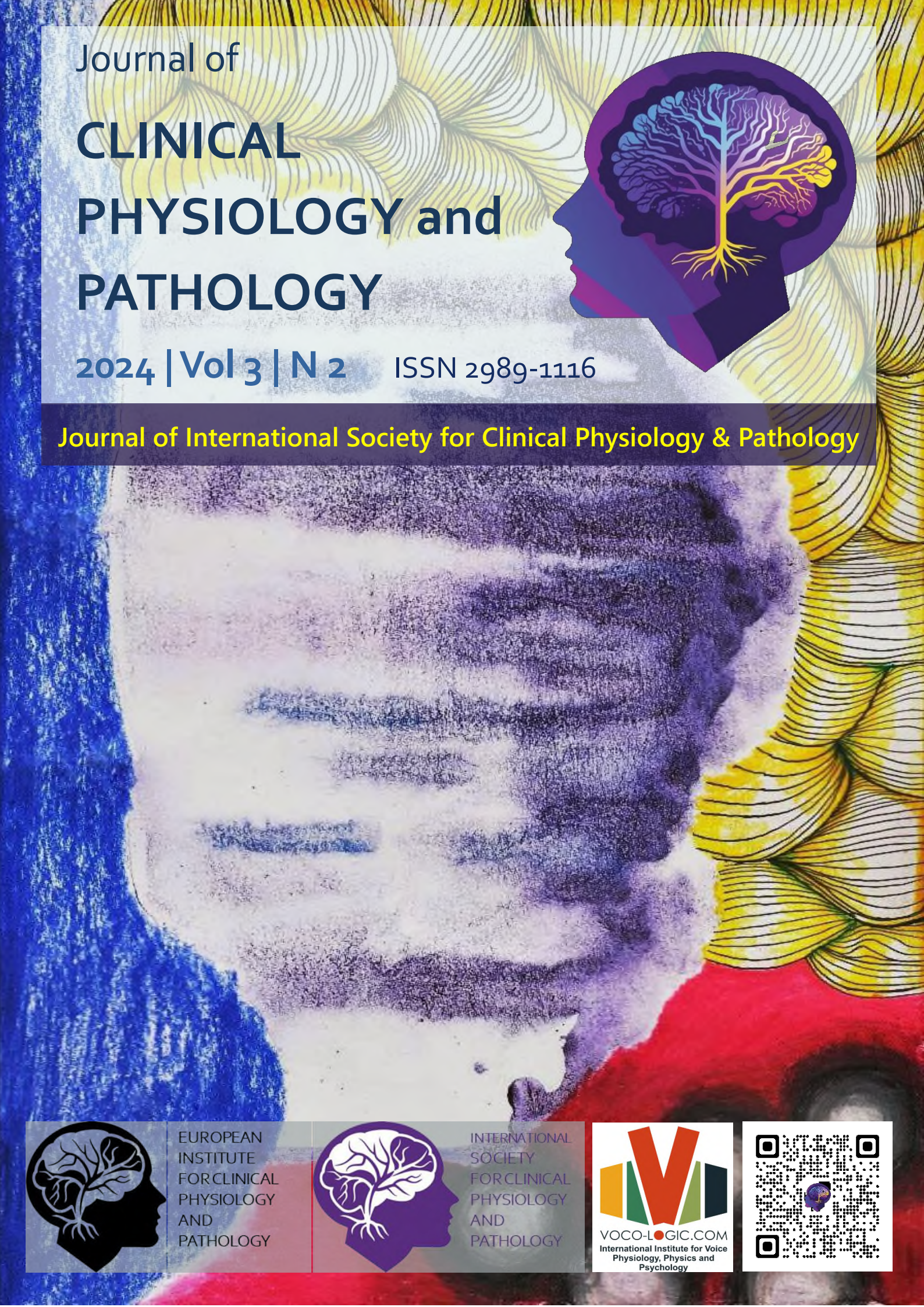
Journal of

CLINICAL PHYSIOLOGY and PATHOLOGY

2024 | Vol 3 | N 2 ISSN 2989-1116

Journal of International Society for Clinical Physiology & Pathology



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Journal of CLINICAL PHYSIOLOGY and PATHOLOGY

2024 | Vol 3 | N 2 ISSN 2989-1116 (Online)

Journal of International Society for Clinical Physiology & Pathology

Medical & biological reviewed journal

The authors declare that they have no competing interests

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Founder and Publisher: **International Society for Clinical Physiology & Pathology**

ISSN 2989-1116 = Journal of Clinical Physiology and Pathology (Online)

COBISS.CG-ID 25476356

Website of ISCPP: https://iscpp.eu/

Website of JISCPP: https://journal.iscpp.eu/

Editor office address: 85347 Norveska, 5, Igalo, Herceg Novi, Montenegro

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Contents

Article

The Comparison of Searching Strategies for Genes Related to Ischemic Stroke: Case-control Human and Model Animal Studies

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Abstract: The genetic basis of ischemic stroke (IS) remains unexplored. In this research we compared the lists of candidate genes obtained with three approaches: classical genome-wide association studies (GWAS), cluster-based GWAS and transfer of transcriptome data from rat to human subjects. The risk genes of IS downloaded from three online repositories were also included into consideration. Human orthologues of rat genes demonstrated good presence in public repositories thus pointing the potentials of rat data transfer approach. Different search strategies resulted in almost unique sets of candidate-genes. We assumed the approaches considered complement each other. The studies of genetic basis of multifactorial diseases can benefit from multiple research strategies.

Keywords: ischemic stroke, genome-wide association studies, animal models, genes

Citation: Khvorykh G., Khrunin A., Filippenkov I., Dergunova L., Limborska S. The Comparison of Searching Strategies for Genes Related to Ischemic Stroke: Case-control Human and Model Animal Studies. Journal of Clinical Physiology and Pathology (JISCPP) 2024; 3 (2): 4-7.

https://doi.org/10.59315.JISCPP.2024-3-2.4-7

Academic Editor: Igor Kastyro

Received: 15.04.24
Revised: 07.05.24
Accepted: 30.05.24
Published: 28.06.24

Publisher's Note: International Society for Clinical Physiology and Pathology (ISCPP) stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Ischemic stroke (IS) is a multifactorial disorder with heritability reaching up to 40% depending on its subtypes [1]. About 80 genes are found to be associated with IS [2] but its genetic basis remains underexplored [3]. The key approaches to identify risk genes are linkage analysis, candidate gene studies and genome-wide association studies (GWAS), among which the last one was the most productive. Nevertheless, it has some limitations, which consequences are incomplete set of genetic markers and low reproducibility. Previously we introduced two promising extensions of GWAS and candidate gene approaches. Firstly, we demonstrated that statistical tests of individual single nucleotide polymorphisms (SNPs) can be elaborated with clustering approaches resulting in blocks of linked SNPs [4]. Secondly, we proposed and applied the protocol for the translation of the results obtained from rat models of IS into humans [5-8]. Here we present the results of comparative analysis of genes obtained with traditional and cluster-based GWAS and with transcriptome analysis of rat brains under ischemic conditions (Figure 1). Risk genes retrieved from three public repositories were also included into comparisons.

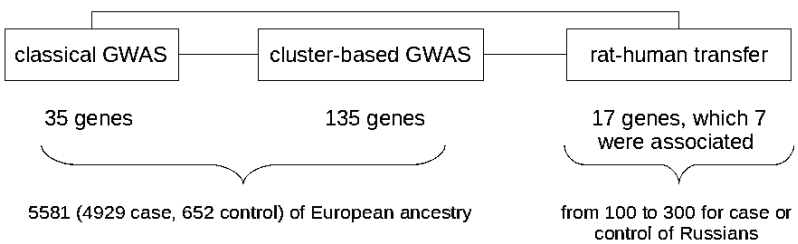

Figure 1. Candidate gene searching strategies

2. Patients and Methods

We explored SNPs in 17 genes obtained by transferring rat genes expressed differentially under tMCAO into human genome, genotyped them by real-time PCR in a cohort of individuals self-identified as Russians [5-8]. Seven of these genes were found to be associated with IS. The classical

and cluster based GWAS were made for 5581 individuals with European ancestry (4929 cases and 652 controls). The first one consisted of statistical testing of individual SNPs under different models of inheritance, the second one utilized SNP grouping with density-based spatial clustering algorithms DBSCAN [9] and HDBSCAN [10] followed by haplotype inference and statistical testing [4]. Previously we did not include the intergenic SNPs in downstream analysis. Now all SNPs were annotated and genes thus obtained considered. The annotation was made with snpEff software [11]. We also considered 131 genes associated with IS from Monarch Initiative (monarchinitiative.org, accessed on 13 June 2023) [12], 1159 genes from DisGeNET (disgenet.org, accessed on 7 October 2022) [13], and 400 genes from GWAS Central (gwascentral.org, accessed on 2 April 2023) [14].

3. Results

The classical GWAS revealed 29 SNPs significantly associated with IS, while cluster-based GWAS detected 666 and 892 SNPs from blocks associated significantly for DBSCAN and HDBSCAN, respectively [4]. The p-values in both approaches were ‹ 0.05 after Bonferroni correction. These SNPs can potentially affect 35 (classical GWAS), 1035 (DBSCAN) and 1362 (HDBSCAN) genes. The number of common genes for both algorithms of clusterization consisted of 135. They were further analyzed. This resulted in 13 common genes between classical and cluster-based approaches. The gene RUNX1 detected with classical GWAS and seven genes (USF1, CD34, KIF26B, MSX2, LHFPL3, RUNX1, and LGALS2) identified with cluster-based approach were presented in online repositories. Seventeen genes analyzed within rat-human approach contained 6 genes (CCL23, HSPB1, PTX3, CD14, LGALS3, and TSPO) from DisGeNET and RGS9 from GWAS Central (Figure 2)

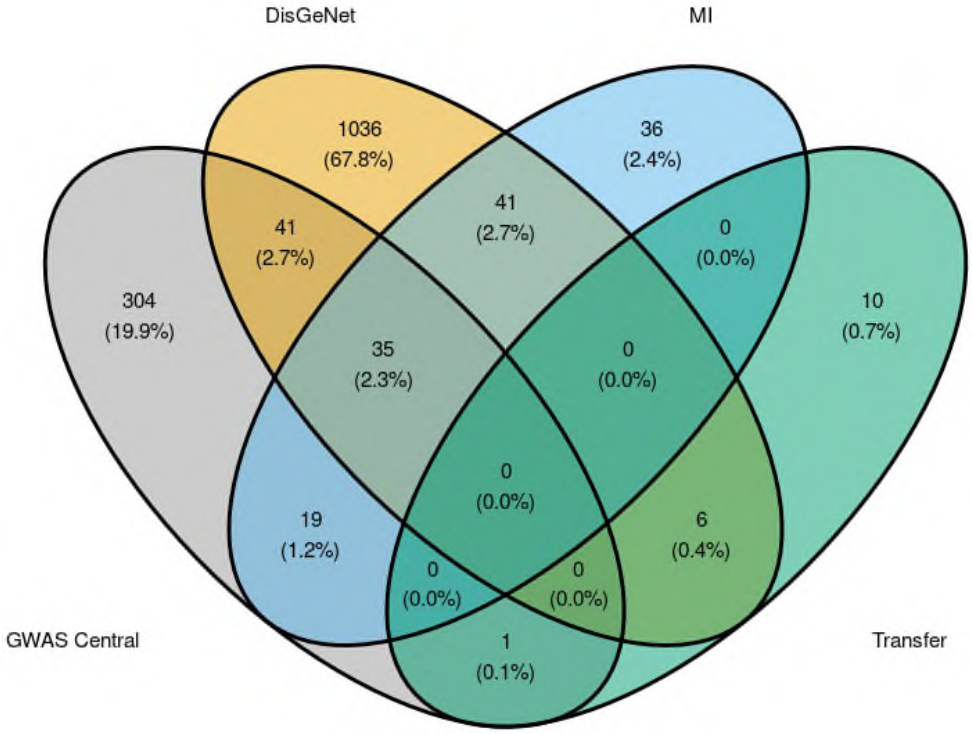

Figure 2 The intersections of candidate genes determined by rat-human transfer ap-proach with known genes in online repositories.

Neither of 7 genes validated by rat-human transfer protocol were presented in the results of classical or cluster-based GWAS. However, two of such genes, LGALS3 and PTX3, were presented in DisGeNET. Among three online resources of genes associated with IS, DisGeNET had the highest number of unique genes, that is 68.6%, while Monarch Initiative has the lowest number (2.4%).

4. Discussion

The number of candidate genes obtained previously for European cohort with cluster-based approach in-creased from 88 to 135 because of inclusion into consideration the intergenic SNPs.

Classical and cluster-based GWAS resulted in 13 common genes, thus demonstrating 14% and 78% of unique genes, respective-ly. We hypothesized cluster-based GWAS detects the genes missed by classical one since it is less influ-enced by multiple testing correction. We saw cluster-based approach resulted in more candidate genes than classical GWAS and it had greater fraction of genes in common with public resources. Therefore, it makes sense to consider the results of both approaches together. Last years, we have elaborated the protocol that allowed transferring the results of transcriptome analysis of rats under model ischemia into human studies. It is interesting to compare the results obtained with human genomic and rat transcriptomic data analysis. Previously we examined 17 human orthologues of rat genes expressed differentially under tMCAO [5-8]. All of these genes except CHRM4 were presented in GWAS data since the SNPs affecting them, according to snpEff, were genotyped and tested. Now we found that seven genes validated with rat-human transfer protocol were not reproduced by classical or cluster-based GWAS. Nine genes that were not verified with rat-human approach were also absent among the significant results of both GWAS approaches. It is clear that this comparison is preliminary, and more genes processed with transfer protocol should be considered. This is supported by the presence of 7 out of 17 genes considered in online repositories as being associated with IS. We believe this indicates a possibility for rat transfer protocol being further applied. For example, other model animals can be analyzed in similar way, allowing new genes associated with IS to be identified.

5. Conclusions

The comparison of three strategies for searching the candidate genes of IS on the level of gene lists showed that they complement each other. A combination of these methods can reinforce the studies of genetic underpinnings of ischemic stroke and other multifactorial diseases.

6. Prognosis and Conclusion

The natural history of Moyamoya disease tends to be progressive in children and adults. In studies with long-term follow-up of untreated patients, progression of neurological deficit and poor outcome were reported in 50-66% of cases. Radiographic progression within five years of diagnosis was noted in 36% of children with moyamoya. Vascular pathology is usually aggravated by extensive occlusion of intracranial large arteries and collateral circulation. Patients often suffer from cognitive and neurological decline due to recurrent ischemic stroke or hemorrhage.

Application of artificial intelligence: The review is written without the use of artificial intelligence technologies.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Funding: This research was supported by the Thematic plan of the National Research Centre "Kurchatov Institute" (5f.5.9.) (gene set comparisons) and by the Russian Science Foundation, grant number 23-14-00131 (per-forming classical and cluster-based GWAS).

Conflicts of Interest: The authors declare no conflict of interest.

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Article

Improvement of Pathogenetic Periodontal Treatment through Laser Combined with EHF Irradiation

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Citation: Parfenova S., Kobzeva Yu., Ostrovskaya L., Domenyuk D., Kochkonyan T., Parfenov A., Aslanyan M., Tverskova V., Rashidova F., Gikoshvili M., Galevich A., Tsaturyan D., Ivanyuta O. Improvement of Pathogenetic Periodontal Treatment through Laser Combined with EHF Irradiation. Journal of Clinical Physiology and Pathology (JISCPP) 2024; 3 (2): 8-12.

https://doi.org/10.59315.JISCPP.2024-3-2.8-12

Academic Editor: Igor Kastyro

Received: 22.04.23
Revised: 10.05.24
Accepted: 05.06.24
Published: 28.06.24

Publisher's Note: International Society for Clinical Physiology and Pathology (ISCPP) stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Rationale. Verification of chronic generalized periodontitis is rated among the most promising areas of personalized therapy. Osteodestructive changes will inevitably lead to tooth loss. Correction of microcirculatory disturbances, which constitute one of the factors indicating the hemostasis of periodontal tissues, may help reduce the financial burden faced by the entire healthcare system when offering medical assistance to the respective category of patients.
Aim of study. This study was aimed at determining the role of combined laser and EHF irradition in the pathogenetic therapy offered to cases of chronic generalized periodontitis.
Materials and methods. The whole set of periodontal treatment procedures offered to 40 patients was expanded with a combination of laser and EHF irradiation (MATRIX unit). As the study was carried out, clinical and laboratory values were recorded, as well as an assessment of the indicators for periodontal tissues was done, namely, measures were taken for the pocket depth; the contents of the pocket discharge was identified; the Muhllemann-Cowell index, the papillary-marginal-alveolar index (PMA), the plaque index (PI), and the oral hygiene index (OHI) were evaluated, along with the hemostasis system microcirculatory link studied – the platelet functional activity (adhesion and aggregation), in particular.
Results. The comprehensive treatment of periodontitis, which included combined laser and EHF irradiation, resulted in an improved clinical status: the depth of periodontal pockets revealed a decrease, whereas no suppuration was to be observed. The improvement in the periodontal tissues came along with positive dynamics of the following indices – the PMA index featured a statistically significant decrease; the PI values showed a change to a lesser extent compared to the PMA index, yet also within the statistically significant range; the oral hygiene improved, which manifested itself through an increase in the OHI values. Notable is that the difference in the index values taken prior to the comprehensive treatment and following it, was significant. This change in the indices is related closely to changes in the aggregation and adhesive capacity of platelets.
Conclusion. Given the above, the obtained data point at high efficiency of the combination of laser and EHF exposure introduced into the set of treatment measures offered for periodontal diseases. The clinical and laboratory data are important both as a theoretical expansion to the available knowledge and from the practical stance. Platelet functional activity indicators are important markers of inflammation issues affecting periodontium. The study outcomes allow viewing the combination of laser and EHF irradiation as an effective component of comprehensive treatment for the said pathology, as well as recommend it be introduced into the treatment plan.

Keywords: chronic generalized periodontitis, combined laser and EHF irradiation, disturbed microcirculation, index evaluation for periodontal tissue status.

1. Introduction

Diseases involving periodontium are one of the most complex pathologies faced by dentists, both in the Russian Federation and abroad, while ranking second in prevalence giving way to dental caries. As reported by the World Health Organization (WHO), 95% of the adult population and 80% of children globally, have been diagnosed with various clinical manifestations of inflammatory periodontal diseases (IPD) [1]. The overall medical and social role of IPD, taken as a special section within dentistry, is due to the following factors: high incidence; diverse etiology; tendency to progression; significant issues in arriving at stable remission; mild symptoms through the early stages; an increase in the number of young capable population featuring severe destructive and atrophic periodontal changes; possible loss of intact teeth; chronic infection foci arising due to the development of gingival and periodontal pockets, as well as their role in the occurrence of general somatic pathology [2-10]. The protective and compensatory periodontal mechanisms and the human body status as a whole is a factor that determines the prevalence and intensity degree of the inflammatory process [11-15].

Gingivitis and periodontitis account for the most common pathologies in the structure of periodontal diseases. The two health issues in question rely on inflammation, a typical pathological process based on changes occurring through the effects of periodontal pathogens [16-18].

The clinical picture of chronic generalized periodontitis in its early stages can be described by a low-manifest and latent course, which makes setting diagnosis in due time complicated and, respectively, leads to a later start of proper treatment and rehabilitation measures [19].

Microcirculation disorders associated with increased activity of the vascular-platelet hemostasis link play a key role in the IPD pathogenesis. Patients with chronic generalized periodontitis have disturbances involving both the aggregation-related function of platelets and their adhesive activity, whereas it is the severity of the disease course that is decisive for the degree of such disturbances [20-24]. One of the most significant effects wrought by helium-neon laser radiation is a positive impact it has on microcirculatory issues. There are results of numerous studies available, which confirm a significant decrease in blood viscosity and platelet aggregation activity. An important point about the hypo-coagulation effect of low-intensity laser radiation is the improvement in the kallikrein-kinin system values [25].

One of the mechanisms behind laser radiation effects is the generation of secondary weak radio emission belonging to the EHF band. A certain part of the biological effects of low-intensity laser radiation may be mediated by this endogenous EHF effect [25]. The advantage of such an impact implies high efficiency, non-invasiveness, none of any adverse responses and contraindications to use, low cost, and good compatibility with other methods [26].

Cells are known to produce electromagnetic vibrations of a very wide range through their life cycle. However, the predominantly narrow range of EHF waves is employed by cells to exchange information required to regulate intracellular functions and intercellular interaction [25,26]. An extra piece of proof to this idea is a response appearing on the side of both cells and the body as a whole, to low-intensity, informational influences [27].

Numerous studies have revealed that the best result when dealing with treating inflammatory periodontal diseases can be expected through combined treatment only, which includes etiological, pathogenetic and symptom-bound therapy [27]. Physiotherapeutic effects serve an extremely valuable component of combined treatment offered to cases of inflammatory periodontal diseases. Effective treatment of microcirculatory disorders determines largely the overall treatment in patients with inflammatory periodontal diseases and has a significant effect on the course of the diseases mentioned above [26]. Treating such disorders via non-medication methods, unlike pharmacotherapy, entails no side effects, and given their significant effectiveness, can be recommended as a component of comprehensive treatment for patients suffering from inflammatory periodontal diseases.

Aim of study

The study was aimed at identifying the role that combined laser and EHF irradiation have in the pathogenetic therapy of chronic generalized periodontitis.

2. Materials and Methods

The examination carried out within the study involved 40 patients with periodontitis, whose comprehensive treatment included the combined effects of laser and EHF irradiation (MATRIX device). Through the study, clinical and laboratory parameters were recorded, as well as an assessment carried out for the indicators showing the status of periodontal tissues: measures were taken for the pocket depth; the contents of the pocket discharge was identified; the Muhllemann-Cowell index, the papillary-marginal-alveolar index (PMA), the plaque index (PI), and the oral hygiene index (OHI) were evaluated, along with the hemostasis system microcirculatory link studied, namely, the platelet functional activity (adhesion and aggregation).

Platelet adhesion and aggregation were evaluated by the impedance method, the principle of which implies recording microcurrents flowing in a special electrode unit when it is immersed in a blood sample. During that, the change in the impedance (resistance) of the electrode system is measured. The impedance increase is in a direct proportion to the platelet mass deposited on the electrode unit. The impedance kinetics allows quantifying the kinetics of the aggregation process. The initial contact of the electrodes with the blood sample results in a platelet monolayer developing on them. Then, as agonists are added (ADP, collagen, arachidonic acid, ristocetin, etc.), there is a gradual aggregation of platelets on the electrodes happening, which leads to some typical changes in the electrical properties of the system.

This method also allows taking into account the leukocyte-platelet adhesion phenomenon observed in some patients' samples. The following parameters were used for quantifying aggregation: the degree of aggregation, which is estimated by the maximum aggregatogram amplitude, which corresponds to the maximum increase in the resistance at the electrode following the introduction of the inductor; the aggregation rate, which is estimated by the aggregatogram amplitude 1 minute following the aggregation start; the delay time – estimated by the time in seconds elapsed after the inductor addition and prior to the start of aggregation registration; the area under the aggregation curve – the product of the amplitude and the rate of its development.

The obtained data statistical processing was performed using the EXCEL and STATISTICA 6.0 software package, with the average value and the average error determined based on the Student and Mann-Whitney reliability criteria.

3. Results

An objective examination of the patients with generalized periodontitis undergoing treatment showed a significant decrease or complete disappearance of inflammation affecting the free and attached gums. Respectively, the depth of periodontal pockets featured a decrease (from 5.28±0.17 mm to 4.04± 0.24 mm), whereas their suppuration was no longer to be observed. Apart from the clinical improvement, there was also positive dynamics registered in the indices. The papillary-marginal-alveolar index demonstrated a statistically significant decrease, if compared to the values obtained prior to the treatment (mild chronic generalized periodontitis cases – by 82.76±3.62%; patients with moderate degree – by 81.1±3.29%; severe cases – by 75.35±2.98%) (Table).

Table 1. Changes in the indices due to the effect of laser and EHF irradiation treatment in patients with periodontitis

Index / Group			Oral hygiene index, points	Papillary-marginal-alveolar index, %	Periodontal index, points
Control (n = 20)			1.03(0.9;1.2)	3.21 (1.1;5.3)	0.032(0.012;0.09)
Chronic generalized periodontitis	Mild course (n=20)	Prior to treatment	2.12 (1.8;2.2) Z1=3.71; p1=0.000205	47.75 (40.1;52.4) Z1=5.17; p1=0.000001	3.47 (2.9;4.1) Z1=4.67; p1=0.000003
		Following treatment	1.17 (0.9;1.4) Z1=1.47; p1=0.140895; Z2=3.92; p2=0.000089	8.23 (4.9;12.6) Z1=2.51; p1=0.012093 Z2=4.67; p2=0.000003	1.74 (1.2;3.5) Z1=2.74; p1=0.006190; Z2=2.05; p2=0.040057
	Moderate course (n=20)	Prior to treatment	2.24(2.1;2.4) Z1=4.33; p1=0.000015	65.6 (51.6;76.8) Z1=5.32; p1=0.000001	4.25 (3.9;4.8) Z1=5.07; p1=0.000001
		Following treatment	1.34 (1.2;1.6) Z1=2.43; p1=0.015247; Z2=3.11; p2=0.001866;	12.41 (5.6;17.1) Z1=3.11; p1=0.001866; Z2=5.18; p2=0.000001	2.25 (1.8;2.6) Z1=2.74; p1=0.006190; Z2=4.00; p2=0.000063

	Severe course (n=20)	Prior to treatment	2.62 (2.5;2.9) Z1=4.29; p1=0.000018	84.4 (78.2;87.3) Z1=6.87; p1=0.000001	6.37 (5.9;7.1) Z1=5.87; p1=0.000001

		Following treatment	1.62(1.5; 1.8)
			Z1=2.63;
			p1=0.008443;
			Z2=3.82;
			p2=0.000136;
				20.8 (17.6;24.2)
				Z1=4.58;
				p1=0.000005;
				Z2=6.68;
				p2=0.000001
					4.13 (3.8;4.5)
					Z1=3.11;
					p1=0.001866;
					Z2=4.58;
					p2=0.000005

Note: each case demonstrates the average value, the lower and the upper quartiles (25%;75%); Z1, p1 – compared with the control group; Z2, p2 – compared with the group of patients prior to treatment.

The periodontal index showed a smaller change (in patients with mild chronic generalized periodontitis – by 49.85±2.08%; in cases of moderate degree – by 47.0±2.31%, whereas patients with severe course featured a decrease of 35.16±1.88%), which, however, fell within the statistically significant range ($p \leq 0.05$). This can be accounted for by the treatment eliminates inflammatory phenomena in periodontal tissues only, yet do not eliminate the periodontal pocket. Along with inflammation subsiding, there was an improvement noted in the hygiene status of the oral cavity, which expressed itself through positive dynamics in the oral hygiene index values (Table). A comparison of the indices before and after the treatment made it obvious that the best clinical results were to be observed after treatment with the MATRIX unit, the difference in the indices being significant ($p \leq 0.05$).

The changes in the indices correlate closely with changes in platelet aggregating and adhesive capacity [26]. The identified correlations of clinical and laboratory data are of importance both in view of theoretical understanding and of practical application. From the theoretical stance, the data explain the mechanism behind pathogenetic changes in periodontal tissues in case of inflammatory diseases. As far as practical use is concerned, platelet functional activity indicators constitute important differential and diagnostic criteria for evaluating inflammatory periodontal diseases [27].

4. Discussion

Comprehensive treatment employing combined laser and EHF irradiation results in a significantly improved clinical course of inflammatory periodontal diseases. This is to be seen from a statistically reliable improvement in the oral hygiene index, the papillary-marginal-alveolar index, as well as the periodontal index. Combined laser and EHF irradiation with a MATRIX device allows putting to a quick stop inflammation in periodontal tissues and prepare patients for the surgical stage of treatment, also preventing complications [28].

The above means that the obtained data serve proof to a high efficiency of combined laser and EHF exposure if used as a pathogenetic therapy aimed at improving the hemostasis system microcirculatory link.

5. Conclusion

When dealing with patients suffering from chronic generalized periodontitis, there is a need to study the aggregation and adhesive activity of platelets, which is to be done through laser aggregatometry.

Combined laser and EHF exposure has a significantly positive effect on the status of the hemostasis system microcirculatory link in patients with chronic generalized periodontitis. The most pronounced effect can be observed in mild and moderate cases.

The high efficiency obtained through combining the effects of laser and EHF irradiation when treating microcirculation disorders is due to an increase in the adhesive and aggregating capacity of platelets, and this allows proposing the said method to be used as part of the comprehensive treatment administered to patients suffering from the respective pathology.

Application of artificial intelligence:

The article is written without the use of artificial intelligence technologies.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

Radiation Methods for Studying the Liver in the Diagnosis of Sinusoidal Obstruction Syndrome in Cancer Patients During Drug Therapy

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Citation: Dunaev A., Bashkov A., Sheikh Zh., Kudryavtseva T., Esin E., Voskanyan S., Shipuleva I., Popov M., Matkevich E., Lazebnaya O. Radiation Methods for Studying the Liver in the Diagnosis of Sinusoidal Obstruction Syndrome in Cancer Patients During Drug Therapy. Journal of Clinical Physiology and Pathology (JISCPP) 2024; 3 (2): 13-15.

https://doi.org/10.59315.JISCPP.2024-3-2.13-15

Academic Editor: Igor Kastyro

Received: 22.04.24
Revised: 07.05.24
Accepted: 03.06.24
Published: 28.06.24

Abstract: Due to the widespread use of drug therapy in the treatment of oncological diseases in the practice of a radiologist, various manifestations of its damaging effects on the liver parenchyma have become more common. One of these side effects is sinusoidal obstruction syndrome, in which a violation of microcirculation develops at the level of the sinuses of the hepatic lobes. Developing pathological changes in the liver parenchyma of a vascular, structural and functional nature can simulate the progression of the oncological process. Also, late diagnosis of drug toxicity can lead to the development of irreversible changes - liver cirrhosis, portal hypertension. Thus, diagnosing sinusoidal obstruction during various medical imaging methods is an urgent task for a radiologist.

Keywords: sinusoidal obstruction syndrome, computed tomography, magnetic resonance imaging, chemotherapy

1. Introduction

Sinusoidal obstruction syndrome (SOS), formerly called veno-occlusive disease, is a life-threatening complication that is associated with high-dose chemotherapy. SOS often develops rapidly and unpredictably. It is important to identify risk factors that will allow timely diagnosis of this complication and initiation of appropriate therapy [1].

It is believed that with SOS there is a toxic effect on the sinusoidal endothelium, endothelial cells, which leads to damage to the sinusoidal barrier [3], giving the liver a bluish tint. This primary damage to the endothelium leads to extravascular release of red blood cells, leukocytes and other blood cells into the space of Disse, which can lead to thrombo-fibrinolytic balance, further dissection of the endothelial lining with embolization and venular occlusion [4]. In the future, hepatorenal hypertension may develop with the development of multiorgan failure.

SOS can manifest itself in acute (1–3 weeks), subacute and chronic phases. Chemotherapy regimens associated with this condition include oxaliplatin, cisplatin, cyclophosphamide and vincristine [2].

Clinically, patients with SOS present with jaundice, hepatomegaly, weight gain, abdominal pain, and encephalopathy [4]. Chronic SOS can progress to liver cirrhosis.

When using instrumental research methods for SOS, hepatosplenomegaly, ascites, edematous thickening of the gallbladder wall, portosystemic shunts and periportal edema are detected. When ascites is detected, it is important to confirm the diagnosis of SOS and carry out a differential diagnosis with malignant ascites associated with the spread of the pathological process through the peritoneum or metastases [7,11]. In surgical patients, the presence of signs of portal hypertension is important because these signs are potentially associated with a worse prognosis for patient survival due to an increased risk of bleeding or liver failure after surgery [6].

2. Diagnostics

Contrast-enhanced CT shows heterogeneous enhancement in the arterial and portal phases of the scan, which is explained by perfusion abnormalities characterized by a "mosaic pattern" or diffuse linear areas of low density resulting from hepatic congestion, which in the delayed phase of scanning may merge with the liver parenchyma [14].

A diffuse reticular pattern in the hepatobiliary phase of contrast-enhanced MRI in patients after chemotherapy is a characteristic feature of TOS [8]. MR images demonstrate a heterogeneous reticular or linear pattern in normal parenchyma, characterized by hypointensity on T1-weighted images and hyperintensity on T2-weighted images. When using a hepatotropic contrast agent, reticular hypointensity of liver tissue on hepatobiliary phase images with a location in the peripheral areas of the liver is highly specific for TOS [12]. This radiological pattern is likely due to decreased penetration of contrast agent into liver tissue due to dysfunctional hepatocyte damage and decreased portal blood flow [13]. In focal SOS, the presence of unclear boundaries, especially on hepatobiliary phase images, as well as the absence of diffusion restriction are important differential diagnostic criteria between toxic manifestations in the liver parenchyma and metastases. Table 1 shows the features of sinusoidal obstruction syndrome [2].

Table 1. Features of sinusoidal obstruction syndrome

Sinusoidal obstruction syndrome occurs when the sinusoidal endothelium of the liver is damaged, usually after administration of oxaliplatin, cyclophosphamide and vincristine. This condition is associated with an increased risk of bleeding and liver failure.	
Radiological findings	**A drug**
Ultrasound: ascites, thickening of the gallbladder wall and hepatosplenomegaly. CT: Ascites, decreased diameter of the right branch of the portal vein (‹0.45 cm), paraesophageal varices, hepatosplenomegaly and recanalization of the umbilical vein. MRI: diffuse hypointense reticular pattern on post-contrast delayed phase hepatobiliary T1-weighted imaging, periportal edema	Oxaliplatin, 6-MP, dacarbazine, azathioprine, cyclophosphamide, fluorouracil and vincristine

3. Results

When conducting oxaliplatin-based chemotherapy, the maximum radiological manifestations of the severity of SOS are determined approximately 4 months after the start of treatment, radiological remission is observed approximately 3 months after cessation of treatment [9]. Cessation of chemotherapy is often accompanied by a decrease in these manifestations, suggesting that SOS, at least in mild to moderate forms, both diffuse and focal, is potentially reversible [10]. Severe forms of SOS can also progress after cessation of therapy, leading to the appearance of regenerative nodules, followed by the formation of cirrhotic changes [15].

4. Conclusions

In conclusion, many chemotherapy drugs can cause various liver lesions in cancer patients, which are becoming more common due to the longer life expectancy of patients. Radiologists must be aware of the imaging features of liver tissue damage during chemotherapy to guide physicians in making therapeutic decisions and, thus, prevent the development of serious complications in patients.

Application of artificial intelligence: The review is written without the use of artificial intelligence technologies.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

Gender Features of Autonomic Regulation of Cardic Activity in Young Athletes

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Citation: Vlasova T., Spirina M., Bezborodova A., Ryzhov A., Tyagusheva E. Gender Features of Autonomic Regulation of Cardic Activity in Young Athletes. Journal of Clinical Physiology and Pathology (JISCPP) 2024; 3 (2): 16-20.

https://doi.org/10.59315.JISCPP.2024-3-2.16-20

Academic Editor: Igor Kastyro

Received: 19.04.23
Revised: 13.05.24
Accepted: 10.06.24
Published: 28.06.24

Publisher's Note: International Society for Clinical Physiology and Pathology (ISCPP) stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Background. The success of sports activities depends on the level of the athlete's functional state. The study of heart rate variability will help determine the adaptive capabilities and state of cardiac reserves in athletes as they currently are, as well as predict sports results. The purpose of this study is to study gender characteristics of the autonomic regulation of cardiac activity in children involved in sports.
Materials and methods. The study involved 22 children aged 12-18 years. All study participants were divided into two groups: group 1 (n=10) – boys and group 2 (n=12) – girls. Heart rate, systolic and diastolic blood pressure, weight and height were measured non-invasively. Based on the data obtained, adaptation potential, body mass index, and Kerdo index were calculated. When assessing heart rate variability, the autonomic balance index (ABI), autonomic rhythm index (ARI) and tension index (TI) of regulatory systems were calculated. Statistical analysis was performed using t-test, U-test and Chi-square test ($\chi 2$).
Results. In both groups, satisfactory adaptation of the cardiovascular system (CVS) to physical activity was noted (BP ‹2.6), but the value of this indicator was 7.25% higher in boys ($p < 0.001$), which indirectly indicates that The functional reserve of the cardiovascular system in adaptation to physical activity is better in girls. When calculating the Kerdo index and studying heart rate variability (HRV), it was found that 50% of boys have sympathetic tone and 10% have parasympathetic tone. In the group of girls, an increase in the tone of the sympathetic division of the ANS occurs significantly more often by 8.3%. The RRNN value in the boys group is 15% higher than in the girls group ($p<0.001$). The NN50 and pNN50 values were also higher among boys by 44.87% and 41.17%, respectively ($p<0.05$). SDNN and RMSSD in the girls group are less by 25.5% ($p<0.01$) and 34.5% ($p<0.05$), respectively. The average heart rate is 11.6% higher among girls ($p<0.01$). IVR, VPR and IN were greater in the girls group by 32.8%, 32.9% and 50.8%, respectively ($p<0.01$).
Conclusions. Satisfactory adaptation of the body to physical activity was evident in both gender groups. Linear rhythmogram and column histogram data show that HRV is higher in the boys group, and the stress index is higher in the girls group.

Keywords: heart rate variability and adaptation, child athletes, Kerdo index, adaptive potential, cardiovascular system.

1. Introduction

Current tasks of sports and the high level of sports achievements indicate the need to study the functional capabilities of the cardiovascular system (CVS), which is a key link in the adaptation of the human body to increased physical activity (PE) [1]. The success of sports activities is directly related to the level of the functional state of the body [2, 3]. The main signs characterizing a high level of the functional state of the cardiovascular system include bradycardia, hypotension and physiological hypertrophy of the myocardium. Rational exercise leads to an improvement in the morphological and functional characteristics of the heart and blood vessels. The heart of an athletic person combines economical activity at rest and the achievement of maximum performance during physical activity [1].

The functional activity of the body is regulated through the joint work of the central nervous system (CNS), immune and endocrine systems. The autonomic nervous system (ANS), which provides adaptive regulation, determines the consistency and, ultimately, the effectiveness of the regulatory systems of the athlete's body [4, 5]. The outcome of exposure to stress factors on the body depends on the level of the functional state of the ANS. Economization and mobilization of func-

tions at rest and during exercise determine adaptive changes in regulatory processes, thereby ensuring variability and variability of regulation under the expected background conditions of activity [6, 7]. The model of heart rate regulation is based on the already studied mechanisms of regulation of the sinoatrial node of the heart. There are autonomous and central regulatory loops. The first of them, autonomous, is formed from the cells of the sinoatrial node themselves with the participation of the parasympathetic division of the ANS. Another circuit, the central one, includes three levels; it provides intrasystem control, hormonal-vegetative homeostasis and interaction of the body as a whole with the external environment [8].

The ANS of adolescent children undergoes a certain transformation due to active hormonal changes. The regulatory functions of the ANS develop unevenly due to the fact that during puberty, significant changes are observed in the ratio both between the severity of sympathetic and parasympathetic, and in the ratio between the segmental and suprasegmental levels of regulation of the activity of the cardiovascular system [9]. During puberty, problems of autonomic nervous regulation arise, such as a decrease in HRV as a consequence of dysregulation of metabolic control [10].

Thus, the autonomic regulation of cardiac activity has gender differences. Assessing the functioning of all regulatory circuits will make it possible to fully determine the adaptive capabilities and state of the athlete's body reserves at the present time and predict the sports result.

2. Patients and Methods

The study was conducted on the basis of the SSOR for cycling in the city of Saransk from September 19, 2022 to October 22, 2022. The study involved 22 children aged 12 to 18 years. All study participants were divided into 2 groups: group 1 (n=10) – boys, group 2 (n=12) – girls. The average age of the participants was 14.9±0.2 years in group 1 and 15.25±0.3 in group 2.

We non-invasively measured heart rate (HR), systolic and diastolic blood pressure (SBP and DBP), body weight, and height. Based on the data obtained, we calculated the adaptation potential (AP) of the CVS using the formula of R. M. Baevsky [11]. Body mass index (BMI) was assessed using SDS tables [12] and centile scales (WHO, 2007). To assess the impact of VNS, the Kerdo index (KI) was used.

Using the BiTronics Lab, a training laboratory for neurotechnology, HRV was assessed using a linear rhythmogram and a column histogram. Short five-minute recordings were used in accordance with the International Standard [13]. The vegetative balance index (ABI), the vegetative rhythm index (VRI) and the tension index of regulatory systems (IN) were calculated.

The distribution of the obtained data corresponds to the law of normal distribution. For indicators for which the differences were not statistically significant, the U test was used. For parametric analysis, t-test and Chi-square test ($\chi 2$) were calculated.

Statistical analysis was performed using the U test and correlation analysis (SPSS Statistics 13) was used.

3. Results and discussion

As part of the study, AP, BMI, KI were calculated, HRV was analyzed and gender differences were identified.

On average, the AP value in group 1 was 1.33±0.016, and in group 2 – 1.24±0.015. In a comparative aspect, the value of this indicator is higher in group 1 by 7.25% ($p<0.001$).

It was found that on average SBP fluctuated within the normal range and amounted to 121.1±0.66 mm Hg in group 1, which is 5.03% more than in group 2 ($p<0.001$). DBP was also normal in both groups and amounted to 78.8±0.37 mm Hg in group 1 and 72±0.57 mm Hg in the 2nd group. In comparative terms, this indicator is higher in group 1 by 9.4% ($p<0.001$). As for KI, its value averaged 30.9±0.99% in group 2, which is 276.1% more than in group 1 (see Table 1). In group 2, increased tone of the sympathetic division of the ANS occurs more often by 8.3% ($p<0.001$).

BMI in group 1 averaged 20.58±0.37 kg/m2, and in group 2 – 20.86±0.22. This indicator does not depend on gender ($p>0.05$), however, it was found that in group 1, deviations in BMI in the direction of increasing and decreasing were observed 3.3% more often than in girls (see Table 1).

Table 1. Distribution of the studied indicators in children of different sexes

Index	Interpretation of the indicator	1st group (boys, n=10)	2nd group (girls, n=12)	χ^2,p
Height, m		1,7±0,01	1,63±0,003	–
Weight, kg		62,14±1,73	56,09±0,65	–

Body mass index, kg/m^2	Average BMI, kg/m^2	20,58±0,37	20,86±0,22	0,65 (p>0,05)
	Body weight deficiency (SDS<-2.0),%	20	16,7	0,94 (p>0,05)
	Normal body weight (SDS±1.0)	60	66,6	
	Excess body weight (+1.0<SDS<+2.0)	20	16,7	
Kerdo index, %	KI medium	11,19±1,91	30,9±0,99	9,16 (p<0,001)
	Parasympathicotonia	10	0	10,6 (p<0,01)
	Normotonia	40	41,7	
	Sympathicotonia	50	58,3	
Adaptive potential		1,33±0,016	1,24±0,015	4,1 (p<0,001)

The indicators used to analyze HRV are presented in Table 2. During the study, we found that RRNN (arithmetic mean of the duration of NN intervals in the analysis epoch) averaged 828±15 ms. in group 1, which is 13% more than in group 2 (p<0.001).

The total number of NN intervals (N) was 183.2±7.25 and 214.5±7.82 among adolescents of the 1st and 2nd groups, respectively. In comparative terms, this indicator is higher by 17.08% in group 1. The number of pairs of studied NN intervals that differ by more than 50 ms. (NN50) in group 1 averaged 48.1±4.4, which is 44.87% more than in group 2 (p<0.05). The proportion of NN50 from the total number of NN intervals (pNN50) in group 1 was 0.24±0.02, and in group 2 it was 0.17±0.018. In comparative terms, this indicator is higher in group 1 by 41.17% (p<0.05). The number of pairs of studied NN intervals that differ by more than 20 ms. (NN20) was also higher in group 1, where this indicator was 82.7±5.64, which is 25.1% more than in group 2 (p<0.05). The standard deviation of mean NN intervals (SDNN) was 149 ± 14 ms. in group 2, which is 25.5% less than in group 1 (p<0.01). The root mean square of successive differences (RMSSD) was 269 ± 20 ms. in group 1 and 200±22 ms. in the 2nd group. In comparative terms, this indicator is higher in group 1 by 34.5% (p<0.05). Heart rate averaged 92.25±1.88 and 103±2.15 for groups 1 and 2, respectively. In comparative terms, the value of this indicator is higher among adolescents of the 2nd group by 11.6% (p<0.01).

In group 2, the average IVR value was 85±4, which is 32.8% more than in group 1 (p<0.01). VPR was also greater in group 2 by 32.9% (p<0.001). The stress index (SI) value was 51.7±0.03.9 and 78.0±5.7 for groups 1 and 2, respectively. Comparing the results obtained, we can conclude that the IN is greater in group 2 by 50.8% (p<0.01).

Table 2. Heart rate variability in children of different sexes

The indicator being studied	1st group (boys, n=10)	2nd group (girls, n=12)	p
RRNN, ms.	828±15	720±10	4,99 (p<0,001)
SDNN, ms.	200±10	149±14	2,96 (p<0,01)
N	183,2±7,25	214,5±7,82	2,94 (p<0,05)
CV, %	30,37±2,09	23,21±2,08	2,43 (p<0,05)
RMSSD, ms.	269±20	200±22	2,32 (p<0,05)

Mo, s.	0,675±0,015	0,597±0,01	4,33 (p<0,001)
AMo, %	0,43±0,019	0,48±0,014	2,12 (p>0,05)
NN50	48,1±4,4	33,2±3,2	2,74 (p<0,05)
pNN50, %	24±2	17±1,8	2,6 (p<0,05)
NN20	82,7±5,64	66,1±5,21	2,16 (p<0,05)
pNN20, %	42±2	36±2,6	1,83 (p>0,05)
HR	92,25±1,88	103±2,15	3,76 (p<0,01)
IVE	64±3,7	85±4	3,85 (p<0,01)
VRI	2,28±0,082	3,03±0,147	4,46 (p<0,001)
VIRS	51,7±3,9	78±5,7	3,81 (p<0,01)

The results obtained showed that both groups had satisfactory adaptation of the cardiovascular system to physical activity (AP <2.6). Since in 1 the AP value is greater than in 2, this may indirectly indicate a better adaptation of the CVS of girls to physical activity.

An KI value in the range from -10 to +10% is considered normal; positive values of this indicator, beyond this range, indicate the predominance of the influences of the sympathetic nervous system, negative values indicate the predominance of the tone of the parasympathetic nervous system. When analyzing the KI values in both groups, it was found that 50% of adolescents in group 1 are sympathicotonic and 10% are parasympathicotonic. In group 2, we revealed a predominance of sympathicotonics.

Considering the fact that parasympathetic influences reduce heart rate and increase RRNN, we can conclude that children of group 1 have a higher tone of the parasympathetic part of the nervous system than children of group 2. A study by R. Abrarov et al [14] showed in a similar group of children the predominance of the sympathetic link in the autonomic regulation of heart rhythm. However, the authors included healthy children in their study, and we included child athletes, so the differences obtained can be explained by the fact that athletes have higher HRV.

The pNN50 indicator is used to assess the predominance of the parasympathetic component of autonomic regulation (VR) over the sympathetic one. Since the value of this indicator is higher in group 1, we conclude that the parasympathetic type of VR is statistically more common in boys.

The SDNN indicator reflects the total effect of the autonomic regulation of the heart and allows us to conclude that in group 1, the predominance of the autonomic regulation of the heart is observed statistically more often. In a study by M. S. Ishbulatova, SDNN increases in different groups of children aged 9-11 years and on average reaches 93.37 ± 10.08 ms. for boys and 81.35±9.2 ms. for girls [15]. Comparing the results of the author's study with ours, we can conclude that in boys the autonomic regulation of heart rate actually predominates, but in our case the value of the studied indicator turned out to be greater, which probably indicates greater HRV in child athletes.

RMSSD is an indicator that allows you to assess the activity of the parasympathetic part of the ANS. This gives us the right to conclude that in children of group 1, the activity of the parasympathetic component of the ANS is statistically more likely to predominate. The obtained result is comparable to the results of studies by other authors. In the same study by M. S. Ishbulatova, RMSSD among boys 9-11 years old was greater compared to girls of the same age and averaged 111.27 ± 14.03 ms. [15].

Taking into account the results obtained, namely the values of RRNN, pNN50, SDNN, RMSSD in both groups, we conclude that adolescents of group 1 have higher HRV than adolescents of group 2.

IVR shows the relationship between the activity of the sympathetic and parasympathetic divisions of the ANS. VPR reflects the balance of regulation of the cardiovascular system by the sympathetic and parasympathetic divisions of the ANS. It is known that an increase in these indicators indicates the predominance of the sympathetic link in the regulation of the ANS. The stress index (SI) indicates the degree of influence of the nervous system on the functioning of the heart.

The calculated indices were lower in group 1, on the basis of which we established that parasympathetic influences predominate in this group and this confirms our conclusion that HRV is greater in group 1.

4. Conclusions

1. In both groups, there is satisfactory adaptation of the cardiovascular system to physical activity, but in girls the AP value is 7.25% less than in boys (p<0.001), which may indirectly indicate better adaptive capabilities of the girls' body.

2. The predominance of the tone of the parasympathetic division of the ANS is typical for the group of boys, where 10% of children are parasympathetic; among girls, an increase in the tone of the sympathetic division of the ANS is more common by 8.3% ($p<0.001$).

3. Taking into account the values of RRNN, pNN50, SDNN, RMSSD, heart rate, as well as the results of the stress index, autonomic rhythm index and autonomic balance index, heart rate variability is higher in the group of boys.

Application of artificial intelligence: The article is written without the use of artificial intelligence technologies.

Author Contributions: Conceptualization, T.V. and M.S.; methodology, T.V. and M.S.; formal analysis, A.B.; investigation, A.B. and E.T.; data curation, A.R..; writing—original draft preparation, A.B., and E.T.; writing—review and editing, M.S. and A.R.; supervision, T.V.; project administration, T.V. All authors have read and agreed to the published version of the manuscript."

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

Computed and Magnetic Resonance Imaging in the Diagnosis of Focal Nodular Hyperplasia in the Liver in Cancer Patients During Chemotherapy

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Citation: Dunaev A., Bashkov A., Sheikh Zh., Kudryavtseva T., Esin E., Voskanyan S., Shipuleva I., Popov M., Matkevich E., Lazebnaya O. Computed and Magnetic Resonance Imaging in the Diagnosis of Focal Nodular Hyperplasia in the Liver in Cancer Patients During Chemotherapy. Journal of Clinical Physiology and Pathology (JISCPP) 2024; 3 (2): 21-23.

https://doi.org/10.59315.JISCPP.2024-3-2.21-23

Academic Editor: Igor Kastyro

Received: 30.04.24
Revised: 17.05.24
Accepted: 05.06.24
Published: 28.06.24

Publisher's Note: International Society for Clinical Physiology and Pathology (ISCPP) stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: The differential diagnosis of focal changes in the liver remains an urgent task for the radiologist. Focal nodular hyperplasia (FNH), being the second most common benign formation of hepatocellular origin, is formed in cancer patients as a result of vascular disorders during chemotherapy, in particular with the development of sinusoidal obstruction. As a result, the resulting FNH node can simulate metastasis in a cancer patient, especially in cases where the primary tumor has a hypervascular structure. However, radiation semiotics based on computed tomography and magnetic resonance imaging data allows, in most cases, to confidently differentiate the nature of the focal formation and avoid false-positive diagnostic results.

Keywords: FNH-like lesions, computed tomography, magnetic resonance imaging, chemotherapy

1. Introduction

Focal nodular hyperplasia is a benign liver lesion consisting of a proliferation of hyperplastic hepatocytes surrounding a central stellate scar. Any new formation in the liver in a patient with a history of cancer raises serious concerns regarding metastasis. After or during chemotherapy treatment, changes such as steatosis, steatohepatitis, and sinusoidal obstruction syndrome may occur in the liver, which may manifest as focal lesions [2]. In addition to manifestations of hepatopathy, against the background of high doses of chemotherapy, benign regenerative lesions in the form of pseudometastatic nodules are detected in the liver [3]. These lesions are considered a late manifestation of sinusoidal obstruction syndrome (SSO). In adults, focal nodular hyperplasia (FNH) develops in patients with colorectal cancer after oxaliplatin-based chemotherapy [4]. The exact pathogenesis leading to the occurrence of FNH after chemotherapy remains unknown. FNH is a benign hyperplastic lesion that occurs in the setting of a vascular malformation and often occurs with a local increase in pressure in the hepatic arteries [5]. An important side effect of treatment regimens using oxaliplatin is the occurrence of sinusoidal obstruction syndrome (SSO), which has a toxic effect on sinusoidal endothelial cells. SSO reduces oxygen saturation of the liver [6], in-

creases the expression of hypoxia-induced factors and stimulates angiogenesis by activating angiogenic factors [7]. It is hypothesized that SSO and associated liver hypoperfusion may lead to the formation of benign regenerative lesions such as FNH.

New or enlarged FNH-like lesions in the liver in patients under observation with a history of cancer prone to metastasis to the liver may lead to unnecessary invasive procedures if they are mistaken for metastases.

2. Diagnostics

On computed tomography (CT), focal nodular hyperplasia classically appears as a homogeneous, isodense, or slightly hypodense lesion relative to the liver parenchyma. On CT with bolus intravenous contrast, the mass appears as a homogeneous hypervascular lesion; In delayed phases of the scan, accumulation of a central scar can be observed. These pathognomonic signs of FNH should be taken into account in the differential diagnosis of hypervascular metastases in the liver. A differential diagnostic sign that distinguishes FNH from metastases is an irregular shape, unclear contours, hypervascular foci in the hepatobiliary phase of scanning on magnetic resonance imaging (MRI). Metastases often accumulate contrast agent in a ring-like pattern.

Additional MRI features characteristic of FNH are signal isointensity on T1- and T2-weighted images, early contrast enhancement of the lesion, and absence of diffusion restriction on DWI (diffusion-weighted imaging) [8].

Metastatic malignancies that are commonly treated with oxaliplatin regimens are most often hypovascular on MRI. Thus, when hypervascular foci appear in the liver in cancer patients during chemotherapy, one should not immediately write about a metastatic lesion; FNH-like benign lesions should be included in the differential list [9].

Table № 1 shows the features of sinusoidal obstruction syndrome [8].

Table 1. Features of sinusoidal obstruction syndrome

Focal nodular hyperplasia is thought to result from microvascular changes in the liver and is associated with oxaliplatin use. Oncologists should be aware of this focal lesion because it can be mistaken for hypervascular metastasis. MRI with hepatospecific contrast agent provides important information for the differential diagnosis of focal nodular hyperplasia.	
Radiological findings	**A drug**
MRI: signal isointensity on T1- and T2-weighted images, early contrast enhancement and no diffusion restriction (DWI)	Oxaliplatin-based chemotherapy

3. Conclusions

Although the appearance of FNH-like lesions in the liver during chemotherapy is rAlthough the appearance of FNH-like lesions in the liver during chemotherapy is rare, it is important for physicians to be aware of the occurrence of these benign lesions because they can simulate metastases and prevent overdiagnosis in such cases and prevent unnecessary invasive procedures.

Application of artificial intelligence: The review is written without the use of artificial intelligence technologies.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

The Influence of the Balance of the Autonomic Nervous System on the Electroencephalographic Activity of the Brain in Healthy Schoolchildren

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Abstract: Objective of the study: Analysis of electroencephalographic data of the brain in healthy schoolchildren depending on the type of autonomic regulation of the body.
Materials and methods: The study involved 12 boys aged 10 to 12 years. Bioelectrical activity of the brain in schoolchildren with different types of autonomic regulation (TAR) was studied using the electroencephalography (EEG) method.
Results: Determination of the types of autonomic regulation and the corresponding EEG indicators made it possible to identify the features of physical adaptation and functional activity during ontogenesis in children under conditions of increased academic loads.

Keywords: electroencephalography, brain, types of autonomic regulation, schoolchildren.

Citation: Gorelik V., Filippova S., Emets Ia., Kastyro I. The Influence of the Balance of the Autonomic Nervous System on the Electroencephalographic Activity of the Brain in Healthy Schoolchildren. Journal of Clinical Physiology and Pathology (JISCPP) 2024; 3 (2): 24-29.

https://doi.org/10.59315.JISCPP.2024-3-2.24-29

Academic Editor: Igor Kastyro

Received: 17.04.24
Revised: 07.05.24
Accepted: 04.06.24
Published: 28.06.24

Publisher's Note: International Society for Clinical Physiology and Pathology (ISCPP) stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

The modern school education system requires a scientifically based approach to the educational process, which should be based on the physiological indicators of the body of schoolchildren in ontogenesis [1, 2]. Children in the process of morphological and functional development are influenced by stress factors of increasing school workload [3,4]. In this regard, the choice of the most optimizing effect of physical education on the adaptation and functional state of children, depending on the types of autonomic regulation, should be based on promising diagnostic methods. the functional state of students who are in real conditions of exposure to constantly increasing learning loads, including information and computer technologies [5, 6]. The purpose of the study. Given the different rates of formation of the bioelectric activity of the brain and the mechanisms of autonomic regulation of cardiac activity in students of the age of second childhood (10-12 years), it seems relevant to study the EEG characteristics of schoolchildren at a certain stage of ontogenesis [2, 8].

2. Material and methods

The study involved 12 boys aged 10-12 years old. The research work was carried out based on MBU "School No. 32" in Tolyatti. The measurements were carried out from September 2024 to November 2024.EEG studies were performed using Neuron-Spektr-4/VPM, a hardware and software complex that combines 21 channels for recording EEG or long-latency evoked brain potentials, 4 polygraphic channels for recording short-latency evoked brain potentials. The heart rate variability (HRV) parameters were evaluated using the Varikard 2.51 software hardware complex to determine the types of autonomic regulation (TAR).

3. Results

For a more detailed study of the mechanisms that form the regulatory basis of the types of autonomic regulation (TAR), the indicators obtained by recording the EEG of the cerebral cortex were studied. The type of EEG recordings of the brain activity of children with different TBR I – II, III, IV presented in Table 1 had the following distinctive features.

Table 1: Assessment of the functional state of regulatory body systems in schoolchildren according to heart rate variability (HRV) [8]

Heart rate regulation type	HRV parameter features according to the dominant regulation type	Interpretation of the HRV data obtained
Moderate predominance of central regulation type I (MPCR)	SI›100 cond. units. VLF›240 ms^2 SI›100 U VLF›240 ms^2	Moderate predominance of sympathetic heart rate regulation
Significant predominance of central regulation type II (SPCR)	SI›100 cond. units. VLF (ms^2) ‹240 SI›100 U VLF (ms2) ‹240	Significant predominance of sympathetic heart rate regulation
Moderate predominance of autonomous regulation type III (MPAR)	SI›70‹150 cond. units. VLF›240 ms^2 SI›70‹150 U VLF›240 ms^2	Moderate predominance of parasympathetic activity
Significant predominance of autonomous regulation type IV (SPAR)	SI‹25 cond. units. VLF›500 ms^2 TP›8000–10 000 SI‹25 U VLF›500 ms^2 TP›8000–10 000	Significant predominance of the parasympathetic department

Note: SI – stress index for regulatory systems, VLF – index (high – hyperadaptive, low – energy deficient state), TP – total power of the HRV spectrum.

In children with I–II TBD (sympathicotonia), a pointed hypersynchronous alpha rhythm was recorded over both hemispheres, which was dominant. The maximum amplitude of the alpha rhythm in the left hemisphere was 136 Mv (average –43 mv), 145 MV above the right hemisphere (average – 41 MV). The interhemispheric asymmetry of the alpha rhythm was 18.4%. The alpha rhythm index over the left hemisphere (on the spectrum) is 41.2%, over the right hemisphere (on the spectrum) – 40.7%. Alpha rhythm prevails in the occipital leads. EEG indicators in students of TAR I–II reflect the typological features of the neural activity of brain structures, namely: a high index of 41.2–40.7% (left/ right, cerebral hemispheres) and the amplitude of activity of a pronounced dominant alpha rhythm, which is formed by the ascending activating effects of the reticular formation and/or thalamic structures on the cerebral cortex. Diagnosed by the method HRV high stress index values express increased activity of the sympathetic division of the autonomic nervous system (VNS). In these children with type I–II TB, the controlled system is destabilized, and dysregulatory manifestations occur in the activity of the central nervous system (CNS). At the same time, the processes of self-regulation are suppressed, adaptive capabilities are reduced to maintain a normal level of functioning. the cardiovascular system, the management of the central nervous system is achieved with significant functional stress on the regulatory systems of the body. Therefore, gymnastic exercises and exercises for developing flexibility, exercises for relaxing muscles, with metered physical activity were offered for these children. Fig. 1, Table 2.

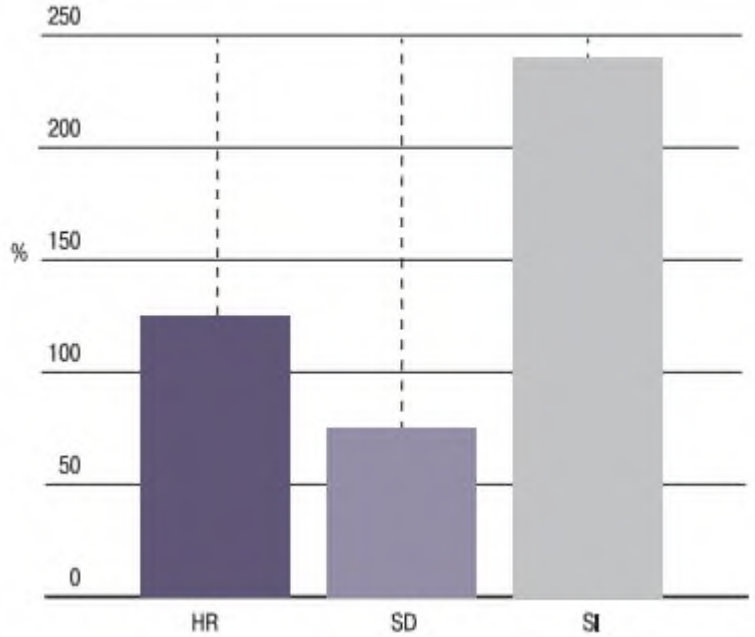

Figure 1. HRV parameters in schoolchildren with TAR I-II (HR – heart rate, SD – standard deviation for cardio-interval, SI – stress index for regulatory systems).

Table 2. HRV and EEG indicators in schoolchildren with different types of vegetative regulation

Vegetative regulation type	SI – stress index, U	Maximum amplitude of the left/right cerebral hemisphere alpha rhythm, μV	Index of the left/right cerebral hemisphere alpha rhythm, %
I–II	297	136; 145	41,2; 40,7
III	140	83; 71	34,3; 24,7
IV	39	32; 35	12,9; 10,6

In the examined children of the III TAR (normotonia), a pointed alpha rhythm was recorded over both hemispheres. The maximum amplitude of the alpha rhythm over the left hemisphere was 83 mv (the average was 24 MV), over the right hemisphere –71 mv (average – 20 MV). Interhemispheric asymmetry of alpharrhythmia is 18.4%. The alpha rhythm index over the left hemisphere (on the spectrum) is 34.3%, over the right hemisphere (on the spectrum) – 24.7%. The alpha rhythm prevailed in the parietal-occipital and posterior temporal leads.

In schoolchildren of the III TAR, at normal values of the voltage of regulatory systems, EEG indicators indicate a moderate dominance of the alpha rhythm, which is the main rhythm of the brain of a healthy person. The alpha rhythm index was determined at the level of 34.3–24.7% (left-right hemisphere), the average value of which was 28%, which is comparable to the theta rhythm index of 18.9%. These data indicate the most optimal interaction between the sympathetic and parasympathetic divisions of the ANS and the central regulatory structures of the brain in the process of regulating heart rhythm. This is a condition of the body It can be taken as a physiological norm of the functional state of regulatory systems reflecting the high adaptive capabilities of the body. In this case, it is the controlled cortical sections of the central nervous system and a balanced system of self-regulation of the central nervous system and ANS that allows you to achieve the optimum cardiac functions during physical exertion of schoolchildren with III TAR without overstrain of the control system of its functional state Fig. 2, Table 2.

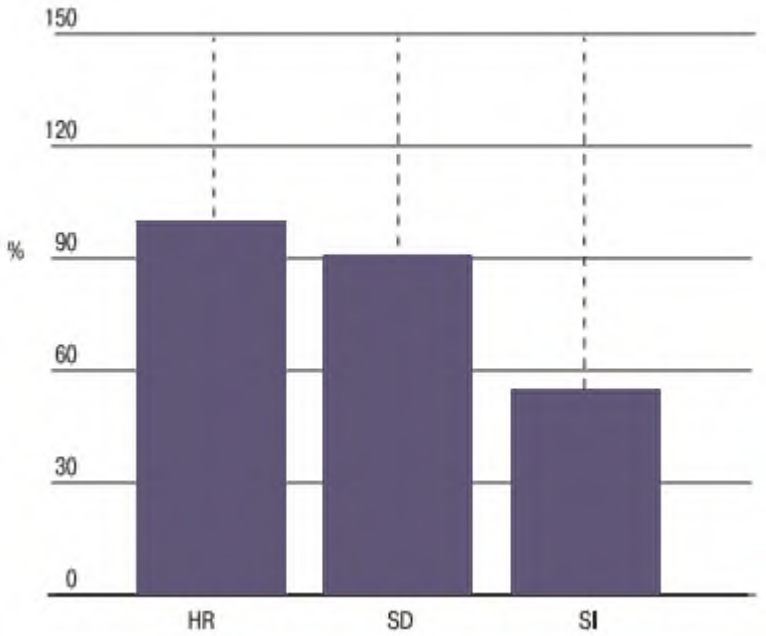

Figure 2. HRV parameters in schoolchildren with TAR III.

In the examined children of type IV TAR (parasympathicotonia), irregular alpharrhythm in combination with theta rhythm was recorded over both hemispheres. The maximum amplitude of the alpha rhythm over the left hemisphere was 32 MV (average – 24 MV), over the right hemisphere – 35 MV (average – 17 MV). The alpha rhythm index over the left hemisphere (on the spectrum) is 10.6%, over the right hemisphere (on the spectrum) – 12.9%. Alpharrhythm prevailed in the posterior temporal and occipital leads. An increase in the slow-wave activity index was recorded over both hemispheres. HRV rates in children with IV The TAR was at the lower limit of the norm or was lowered. In children with type IV TBD, there was a pronounced predominance of autonomous regulation compared with types I, II, and III , the lowest heart rate, the highest duration of cardiac intervals (R–R), the spread of cardiac intervals (MxDMn), and low values of the Si stress index were found (Fig. 3).

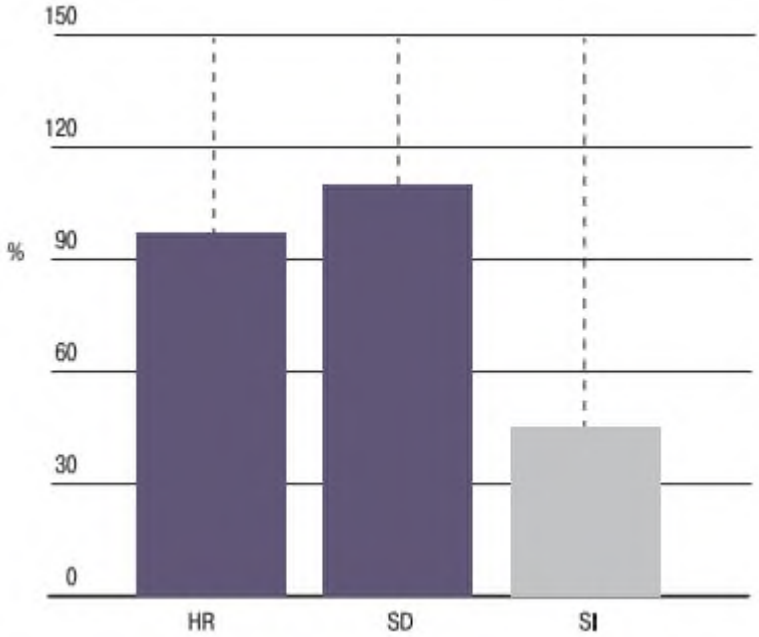

Figure 3. HRV parameters in schoolchildren with TAR IV.

These children often have arrhythmias of various etiologies, fatigue, which can be interpreted as an imperfection of regulatory mechanisms. In this regard, outdoor games and relay races with a metered load were proposed for them. (Fig. 3, Table 2). Thus, the study showed that there are characteristic rhythmological differences in the EEG pattern in children with different types of

autonomic regulation, which allows us to judge the features of brain electrogenesis in schoolchildren. That is, the neuroregulatory activity of the central regulatory circuit controls genetically determined combinations of functional parameters of the ANS departments.

3. Discussion

As noted in the work of D.B. Demin, L.V. Poskotinov, E.V. Krivonogova [2], especially in sympathotonics, higher background activity of subcortical diencephalic brain structures was noted. The "maturation" of the EEG wave structure is accompanied by an increased frequency of hypersynchronous, high-amplitude EEG variants, which is consistent with the data we have obtained. The formation of hypersynchronous EEG patterns in schoolchildren with an increase in sympathetic activity indicates the presence of dysfunctions of the diencephalic structures of the brain and damage to thalamocortical connections, which may underlie a violation of the central mechanisms of regulation of vascular tone. It is known that a controlled increase in the activity of the parasympathetic department of autonomic regulation can lead to an improvement in the state of cerebral blood flow and bioelectric processes of the brain [10]. In children, mainly from groups with a balanced vegetative tone, after performing corrective physical exercises, rhythms are regulated and bioelectric activity shifts towards higher amplitudes in the alpha range, which may indicate a decrease in the level of emotional stress during task performance and synchronization of cortical-subcortical interactions [11].

EEG indicators in students with parasympathetic activity are characterized by a predominance of slow-wave activity reflecting the deep structures of the brain, being an indicator of the mesolimbic effect on the cerebral cortex associated with the emotional state [12].

The results obtained indicate the need for a differentiated approach in physical education classes for children with different types of autonomic regulation, confirmed by the individual characteristics of EEG indicators. HRV also reflects the tension of the functional systems responsible for regulating the body during stress [13-17]. The HRV frequency spectrum fully reflects changes in the sympathetic and parasympathetic divisions of the ANS [18, 19], which must be taken into account when analyzing the health of schoolchildren after physical exertion. The proposed equal workload for children with different TVD can lead to dysregulatory manifestations and a decrease in the adaptive capabilities of students [9]. Building a trajectory of physical education classes based on the typology of the child's body and confirmed by EEG indicators will help strengthen the body of schoolchildren and preserve their health.

4. Conclusions

The EEG study determined the parameters of brain electrogenesis in schoolchildren with various types of cardiovascular system functions.

It has been established that the rhythmological differences in the EEG in children with different TAR correspond to the characteristic features of TAR and can be considered as selective neuro-regulatory influences of the central regulatory circuit on the ANS departments, depending on the genetically determined regulatory typology.

Application of artificial intelligence: The review is written without the use of artificial intelligence technologies.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

Morphological Changes of the Recipient Site During Autotranplantation of Fat in Rats after Various Methods of Its Mechanical Processing

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Citation: Kostyaeva M., Moroz S., Lavrenteva E., Khlystalov M., Ibadullaeva S., Ivanova Y. Morphological changes of the recipient site during autotransplantation of fat in rats after various methods of its mechanical processing. Journal of Clinical Physiology and Pathology (JISCPP) 2024; 3 (2): 30-35.

https://doi.org/JISCPP.2024-3-2.30-35

Academic Editor: Igor Kastyro

Received: 11.04.24
Revised: 29.04.24
Accepted: 19.05.24
Published: 28.06.24

Publisher's Note: International Society for Clinical Physiology and Pathology (ISCPP) stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Introduction. Autologous fat transplantation is a widely used technique in aesthetic and reconstructive surgery, employed for correcting atrophic changes and scar deformities.

Materials and methods. The animals were divided into 5 groups, with 6 individuals in each group. Group 1 – intact animals (without exposure); group 2 – control (saline solution); group 3 consisted of animals implanted subdermally, through an incision with a diameter of 1 mm, a fragment of fat previously extracted from a skin incision in the groin area. In group 4, fat crushed with a scalpel was injected under the skin with a syringe, once. In group 5, animals were implanted with fat crushed using a shredder nozzle in a Luer Lock syringe. 4 implantations were performed, with a volume of 0.05 ml. The sections were stained using the Mallory method, hematoxylin and eosin, methylene blue.

Results. Morphometric parameters of the dermis indicate an increase in its thickness, mainly of the mesh layer, as well as, physiologically significant, an increase in the proportion of microcirculatory vessels in the skin of animals of the experimental groups. The number of sebaceous gland profiles in the experimental groups was slightly higher than in the control groups ($p<0.05$). The thickness of the fat autograft was significantly greater in the third group ($p<0.001$), where a large fat fragment was implanted, however, in this group, almost all rats had pathological reactions in the form of leukocyte, mainly lymphocytic, infiltrations and necrosis of varying severity. Infiltrations containing lymphocytes and fibroblast-like cells were also observed in animals of the fourth and fifth groups, but were significantly less pronounced than in the third. In animals of the fifth group, relatively large fat fragments surrounded by lymphocytic infiltrates were detected only in the skin of two rats, in other animals, fat fragments of microscopic size were found, which, as a rule, were integrated with the adipose tissue of the transplant site.

Conclusion. The introduction of autologous fat can not only lead to its gradual degradation, but also stimulate the formation of new adipose tissue in the injection area. The components of the fat graft (lipids) are incorporated into the newly formed adipose tissue. This process is more effective the smaller the size of the injected graft particles. A significant factor, largely determining the effectiveness of the process, is the stimulation of angiogenesis, which is evidenced by an increase in the number of microvessel profiles in the section and their greater volumetric proportion in the newly formed connective tissue

Keywords: autofat, inflammation, fat graft, fat graft modeling.

1. Introduction

Autologous fat transplantation is a widely used technique in aesthetic and reconstructive surgery, employed for correcting atrophic changes and scar deformities [1]. The main disadvantage of artificial fillers and implants is that they are non-biological substrates and often provoke implant rejection, contour deformities, implant migration, and capsular contractures [2]. Adipose tissue is a biologically active substance, and its function extends far beyond fat storage. It, or rather its stromal-vascular fraction, is one of the main sources of mesenchymal stem cells (MSCs). The multipotency of these cells manifests, in particular, in their ability to differentiate into adipocytes, osteocytes, chondrocytes, and other cells of mesenchymal origin [3]. Adipose tissue is a more accessible source of cellular material than red bone marrow cells [4]. Currently, the effect of MSCs on the stimulation of angiogenesis, remodeling of fibrous tissue, and the possibility of implementing these biological effects in clinical practice through autologous fat transplantation for regenerative purposes in both plastic surgery and related specialties are being actively studied [5].

The aim of this study was to investigate the histological changes in autologous fat grafts and surrounding tissues in rats after various fat processing methods, 30 days after surgical interventions.

2. Patients and Methods

A comparative histological study was conducted on male inbred Wistar rats, 3-4 months old, weighing 195±25 g. The animals were divided into 5 groups, with 6 individuals in each group. Group 1 – intact animals (without any intervention); Group 2 – control group (physiological saline); Group 3 consisted of animals that received a subdermal implant, through a 1 mm incision, of a fat fragment previously extracted from a skin incision in the inguinal region. In Group 4, fat minced with a scalpel was injected under the skin using a syringe, in a single dose. In Group 5, animals were implanted with fat minced using a mincing nozzle in a Luer Lock syringe. Four implantations were performed, with a volume of 0.05 ml each. The material was injected into the interscapular region of the back, slightly lifting the skin for subdermal administration in a 1 cm2 area. After 1 month, the animals were euthanized using a toxic dose of Zoletil 100 solution. For histological examination, a 4 cm2 skin flap was taken and fixed in 10% neutral formalin. Fixation, preparation, and staining of the samples were carried out according to classical methods. The sections were stained using Mallory's method, hematoxylin and eosin, and methylene blue. An Axiostar light microscope (Carl Zeiss) was used in the study. During histological examination, the characteristics of the epidermis, dermis, and subcutaneous fat layer were taken into account, and the specific proportion of microcirculatory vessels was assessed. One section from each animal in the group was examined. Measurements were performed at a maximum magnification of 400, in fields of view where vessels were present. The total area of microcirculatory vessels in the dermis was measured, and then the total area of the dermis in the field of view was measured. The calculation was performed using the formula:

$P = \Delta Sc/Sd.$

P - the specific proportion of vessels (%), Sc - the total area of vessels in the field of view, Sd - the area of the dermis in the field of view.

Morphometric studies were performed using an ocular micrometer. In each sample, 10 measurements of the epidermis and 10 measurements of the dermis were made. Then the average value was calculated. The epidermis was measured from the stratum corneum to the basement membrane, excluding areas with a hair follicle funnel.

The results were processed using the "Microsoft Excel" version 2010 statistical software package. To assess the statistical significance of differences between groups, the following values were calculated: sample mean ( ), difference of sample means ( ), standard error of the difference of sample means ( ), and a 95% confidence interval (95% CI) for the difference of means. A critical significance level of p=0.05 was considered.

Animal studies were conducted in accordance with the requirements of the Order of the Ministry of Higher and Secondary Special Education of the USSR No. 742 of November 13, 1984, "Rules for Conducting Work Using Experimental Animals".

3. Results

Table 1 data indicate the state of the epidermis, where an increase in the thickness of the cellular epidermal layers occurs, mainly due to an increase in the rows of cells in the granular layer. This is particularly evident in the skin of rats in the fourth group ($p<0.01$). During the study, an increase in the rows of cells in the spinous layer and mitotic figures (metaphase plates) ($p<0.01$) were also noted in the skin samples of the experimental animals.

Table 1. Values of cellular epidermis layer thickness 30 days after fat tissue transplantation.

Parameter (microns)	1 group	2 group	3 group	4 group	5 group
Thickness of the cellular epidermis	16,6±0,54	16,2±0,63	17,9±0,45	18,2±0,67	17,6±0,33
Thickness of the granular layer	5,2±0,47	5,0±0,33	6,5±0,38	7,2±0,41	6,9±0,33

Morphometric parameters of the dermis (Table 2) indicate an increase in its thickness, mainly in the reticular layer, as well as, which is physiologically significant, an increase in the proportion of microcirculatory vessels in the skin of animals in the experimental groups ($p<0.001$). However, no significant differences were observed between the groups regarding the area of sebaceous glands. The number of sebaceous gland profiles in the experimental groups was slightly higher than in the control group ($p<0.05$). The thickness of the fat autograft was significantly greater in the third group ($p<0.001$), where a large fat fragment was implanted, but in this group, almost all rats showed pathological reactions in the form of leukocyte, mainly lymphocytic, infiltrations and necrosis of varying severity (Fig. 1).

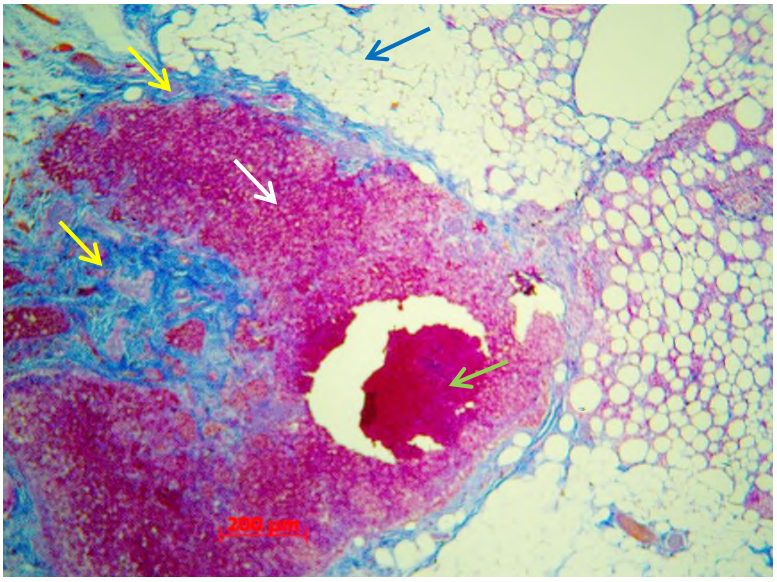

Figure 1. Changes in the fat graft area in group 3. Okr. according to Mallory, uv. x 100. Note: necrosis site (green arrow), lymphocytic shaft (white arrow), connective tissue (yellow arrows).

Table 2. Morphometric parameters of dermis thickness, fat graft thickness, and dermis structure status 30 days after fat tissue transplantation.

Parameter	Thickness of the dermis, microns	Thickness of the fat implant, microns	Sebaceous gland area, mm2	The proportion of vessels of the microcirculatory bed in %	Parameter
Group 1	348±32	0	4965,6±459	5,06±1,14	Group 1

Group 2	380±41	0	5120,5±637	6,05±1,22	Group 2
Group 3	510±56	700±86	4945,6±417	8,05±0,99	Group 3
Group 4	630±44	490±33	5642,6±233	9,45±1,03	Group 4
Group 5	590±22	450±27	5324,9±146	8,65±1,07	Group 5

In animals of the fourth and fifth groups, infiltrations containing lymphocytes and fibroblast-like cells were also observed, but were significantly less pronounced than in the third group ($p<0.01$). This may be due to the significant size of the fat graft, which mechanically damages the surrounding tissues, and a longer period of its breakdown into smaller fragments. In the animals of the fifth group, which received an implant of fat minced using a special nozzle, relatively large fat fragments surrounded by lymphocytic infiltrates were detected only in the skin of two rats; in the remaining animals, microscopic fat fragments were found, which, as a rule, were integrated with the adipose tissue at the transplantation site.

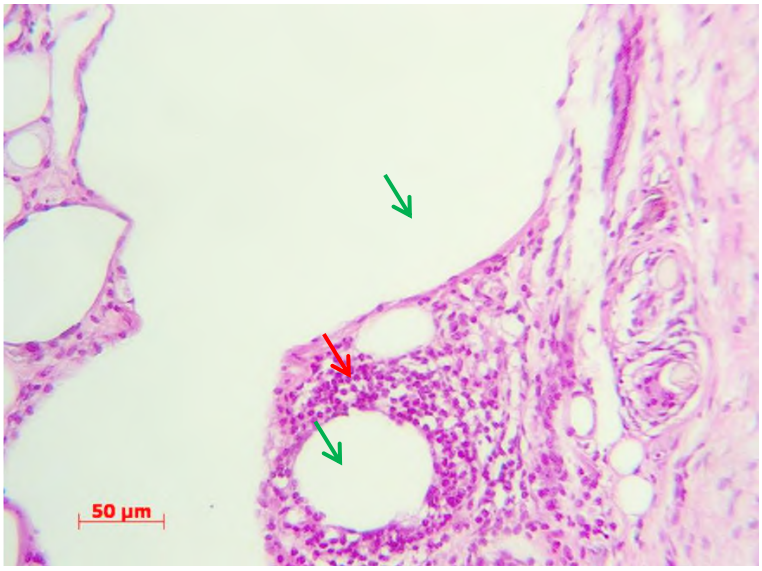

Figure 2. Changes in the area of homogenized fat transplantation in group 5. Ocd. hematoxylin and eosin, uv. x 100. Note: fat fragments (green arrows), infiltration (red arrow).

In the fourth group, where fat minced with a scalpel was implanted once, similar to the fifth group, relatively large rounded fat fragments were found in only two animals. These fragments, as in the previous group, were accompanied by moderate lymphocytic infiltration and limited focal tissue necrosis. In the remaining four rats, fragments of the fat graft were not found in the skin sections at all. However, in the area where the subcutaneous tissue is usually located, between the reticular layer of the dermis and the subcutaneous muscle, rather extensive accumulations of adipose tissue cells were regularly found.

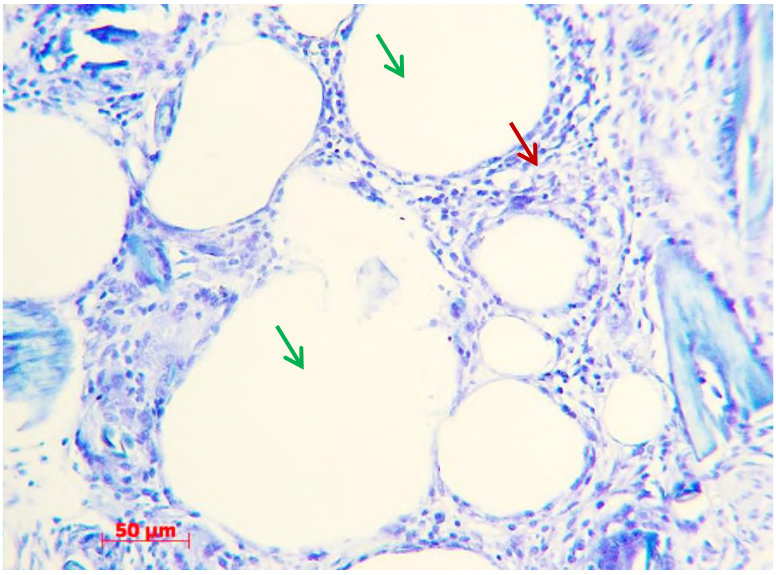

Figure 3. Changes in the area of the fat graft in group 4. Okr. methylene blue, uv. x200. Note: fragments of fat (green arrows), infiltrate (red arrow).

4. Discussion

In accordance with the aim and objectives of this work and the data obtained from the experimental study, it is advisable to discuss issues concerning changes in the morphological parameters of the skin of rats that underwent autologous fat transplantation in the form of fat fragments (grafts) of various sizes. The conditions of the study were standardized as much as possible in terms of the type, age, and weight of the experimental animals, their housing method, and their distribution into groups. To study the effects of autologous fat grafts of different sizes, the animals were divided into five groups.

Numerous studies have shown that adipose tissue is a highly biologically active type of connective tissue. Its stromal-vascular fraction contains multipotent MSCs and a large population of progenitor cells, including adipocyte precursors [6]. The capabilities of MSCs in adipose tissue extend beyond local effects – stimulation of angiogenesis, remodeling of fibrous tissue, stimulation of wound healing, modulation of inflammatory and immune responses, and others.

5. Conclusions

Our studies indicate that the introduction of autologous fat can not only lead to its gradual degradation, but also stimulate the formation of new adipose tissue in the injection area. The components of the fat graft (lipids) are incorporated into the newly formed adipose tissue. This process is more effective the smaller the size of the injected graft particles.

A significant factor, largely determining the effectiveness of the process, is the stimulation of angiogenesis, which is evidenced by an increase in the number of microvessel profiles in the section and their greater volumetric proportion in the newly formed connective tissue.

Author Contributions: Conceptualization, M.G. Kostyaeva; data collection, M.G. Kostyaeva, and E.A. Lavrenteva; data analysis, M.V. Khlystalov, S.S. Ibadullaeva, and Y.V. Ivanova; writing—original draft and visualization, M.G. Kostyaeva, and S.E. Moroz; writing—review and editing, M.G. Kostyaeva. All authors have read and agreed to the published version of the manuscript."

Application of artificial intelligence: The review is written without the use of artificial intelligence technologies.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

Dynamics of Cytokine Concentration in the Wound Infiltrate of the Periodontal Pocket in the Treatment of Endodonto-periodontal Lesions

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Abstract: For the first time, the dynamics of the content of cytokines IL–1a and IL–1b at the stages of treatment of endodonto-periodontal lesions and in the long-term period was studied. The trend of concentration interleukin concentration dynamics for 6 months was evaluated. It was found that the inclusion of an ozone-air mixture in the complex treatment of endodonto-periodontal lesions can increase the effectiveness of treatment of a group of patients with this pathology, which is confirmed by clinical studies.

Keywords: endodonto-periodontal lesions, cytokines, ozone-air mixture..

Citation: Grudyanov A., Kichenko S., Makeeva M. Dynamics of Cytokine Concentration in the Wound Infiltrate of the Periodontal Pocket in the Treatment of Endodonto-periodontal Lesions. Journal of Clinical Physiology and Pathology (JISCPP) 2024; 3 (2): 36-40.

https://doi.org/JISCPP.2024-3-2.36-40

Academic Editor: Igor Kastyro

Received: 15.04.24
Revised: 07.05.24
Accepted: 30.05.24
Published: 28.06.24

Publisher's Note: International Society for Clinical Physiology and Pathology (ISCPP) stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

The urgency of the problem of endodonto-periodontal lesions (EPL) is associated with the complexity of diagnosis and treatment of patients with combined periodontal pathology and complications of dental caries (pulpitis and periodontitis). Insufficient awareness of doctors about the features of these lesions and their complex treatment, along with weak interdisciplinary interaction between specialists of various specialties, explains the overall low effectiveness of treatment in this group of patients. Treatment of patients with EPL requires a one-time elimination of infection from the root canal system and periodontal pocket, and the relief of periodontal inflammation is more difficult [3]. Today, EPL is divided into two broad groups: periodontal lesions with secondary pulp involvement (PVE) and endodontic lesions with secondary periodontal involvement (EVP). The role of local non-specific protection factors in periodontitis (phagocytic cells, complement system, cytokines) was studied by G. M. Barer and T. I. Lemetskaya (1996) [1,2]. Many researchers believe that cytokine imbalance is the basis for the development of chronic inflammatory reactions, so it is cytokines that have a high diagnostic and prognostic value [4,5,6,8,9].

The cytokine IL–1 is the main proinflammatory cytokine that stimulates the proliferation and differentiation of various cell types. Two types of IL–1 are known: IL–1a and IL–1b. IL–1a is a mediator of short–acting action, located mainly inside the cell, upon destruction of which it is released. IL–1b is a secretory form that can act locally and systemically. Being an autostimulator, it plays a key role in triggering the cytokine pro-inflammatory cascade, supporting the inflammatory process and further destruction of alveolar bone tissue. [7,8].

The aim of this study was to assess the possibility of improving the effectiveness of EPP treatment with the inclusion of an ozone-air mixture in the complex of therapeutic measures based on the analysis of the dynamics of the content of cytokines IL–1a and IL–1b.

2. Materials and Methods

The material was collected from patients of the comparison group (20 people) and the observation group (20 people) using strips of sterile filter paper 10x4 mm in size, immersing them in the periodontal pocket for 1 minute (time was measured using an hourglass counted for 1 minute) (Fig. 1), then the paper was immersed in the periodontal pocket.

Figure 1. The extraction of wound exudate from the periodontal pocket with a strip of sterile filter paper.

An Eppendorf tube with a capacity of 1.5 mm containing 100 mcl of sterile saline solution, the volume of the solution was measured using an insulin syringe (Fig. 2). The extraction of wound exudate from the periodontal pocket was performed before treatment and 1, 3 and 6 months after treatment

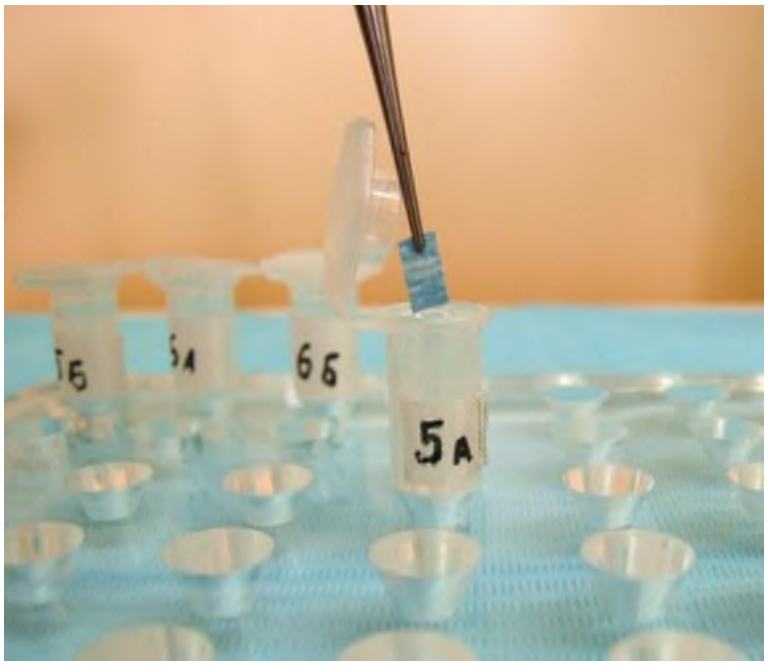

Figure 2. A strip of filter paper in an Eppendorf tube

The release of interleukins 1a and 1b was determined by enzyme immunoassay (ELISA). EIA is an immunological method for the determination of various compounds in biological fluids based on an antigen-antibody reaction. A solid—phase version of the EIA test was used, when one of the components of the immune response was absorbed on a solid carrier and commercial reagent kits Human IL-1beta Platinum ELISA and Human IL-1alfa Platinum ELISA (eBioscience, USA).

The results were recorded using a PR 3100 Bio Rad spectrophotometer (Bio-Rad Laboratories, USA), which measures the optical density of samples at a wavelength of 450 nm. According to the manufacturer, for IL-1a reagents, the sensitivity is 1.1 pg/ml, the range of the calibration curve

of the standards is 1.6–100 pg/ml; for IL-1b reagents, the sensitivity is 0.3 pg/ml, the range of the calibration curve of the standards is 3.9-250 pg/ml.

The results of the studies were processed by methods of variation statistics using the Microsoft Excel program, with an optimistically reliable analysis of the differences in indicators at the significance level p<0.05.

3. Results

The average values concentration of IL-1b concentration in the wound exudate of the periodontal pocket are presented in Tables 1 and 2.

Table 1. Concentration of IL-1β in patients with EVP at the treatment stages

Group Terms	Average values (pg/ ml)	
	Comparison	Group
Pre-treatment	Follow-up group 108.5±38.64,64	117.4,±23.7373
Student	t=-0.197, p=0.848	
1 month	28.26±14.28	76.76±33.81
Student	t=-1.322, p=0.216	
3 months	34.4±7.006	43.57±32.84
Student	t=0.273, p=0.79	
6 months	39.0±17.59	66.53±10.37
Student	t=1.348, p=0.207	

Note: The difference between the groups is not statistically significant, p>0.05.

Table 2. Concentration of IL-1b in patients with PVE at the stages of treatment

Group Terms	Average values (pg/ ml)	
	Comparison	Group
Pre-treatment	Follow-up group 104.5±30.69	144.7±34.26
Student	t=-0.873.873, p=0.403403	
1 month	47.57±17.43	89.48±33.31
Student	t=-1.115, p=0.291	
3 months	69.53±30.55	73.65±19.63
Student	t=0.113, p=0.912	
6 months	87.07±36.18	96.7±34.39
Student	t=-0.193, p=0.851	
Student	t=1.348, p=0.207	

Note: The difference between the groups is not statistically significant, p>0.05.

There were no statistically significant differences in the decrease in IL-1b concentration between the groups, which is most likely due to the small number of observations (EPP is a relatively rare pathology) as well as a significant variation in IL-1b concentrations before treatment. Within the group, there was a noticeable decrease in the concentration of interleukins at various times after treatment.

Since, due to the small number of observations on the concentration of IL-1b, the studied samples turned out to be unrepresentative, an assessment of the trend in the dynamics of interleukin concentrations over the entire observation period (6 months) was carried out.A decrease in their concentration by more than 33.3% was recognized as a positive sign characterizing a decrease in the inflammatory response in periodontal tissues. (Table 3)

Table 3. Trends in decreasing IL-1b concentration in the wound exudation of the periodontal pocket

	EVP	PVE

time frame	Comparison Group	Monitoring Group	Comparison Group	Monitoring Group
0-6 months	36.6%	86.7%	20%	43.3%
Z-score	z=3.725, p=0.00		t=1.663, p=0.11	

The results of statistical processing using the Z-score showed that in patients with EVP lesions in the observation group, the concentration of IL-1b decreased significantly more intensively than in the comparison group. The intensity of the decrease in IL-1b concentration **y** in patients with PVE lesions in the observation and comparison groups didn't significantly differ from each other, since PVE lesions, when the primary source of infection is the microflora of the periodontal pocket, are more severe and after treatment they have deeper periodontal pockets compared to the group of patients with EVP. Therefore, despite the clinical success of treatment, interleukin values indicate the presence of a more pronounced local immune response of eta to the biofilm microflora compared to the EVP group.

The method of data processing using the z criterion revealed that although the absolute values of the average concentration in the comparison group were lower than in the observation group, only two patients showed a decrease in concentration at all terms, while the rest showed abrupt changes both in the direction of decrease and increase. Thus, treatment in the observation group should be considered more effective.

When comparing the average values of IL-1a concentrations, statistically significant differences were obtained between the groups at 3 and 6 months in patients with EVP (Table 4).

Table 4. Concentration of IL-1a in patients with EVP at the stages of treatment

Group Terms	Average values (pg/ml)VP	
	Comparison	Monitoring
Pre-treatment	170.8±41.64	101.9±24.67
Student	t=-1.422, p=0.185	
1 month	104.1±19.31	51.35±23.33
Student	t=-1.742, p=0.112	
3 months	43.82±10.76*	10.15±2.687*
Student	t=-3.038, p=0.013	
6 months	14.33±3.89*	5.9±1.68 *
Student	t=-1.962, p=0.078	

Note: *The values are statistically significant.

The presence of a statistical model of overness already at the average level allowed us to abandon the analysis of trends for this interleukin.

Table 5. Concentration of IL-1a in patients with PVE during treatment stages

Group Terms	Average values (pg/ ml)	
	Comparison	Monitoring
Pre-treatment	62.8±27.69	72.95±25.59
Student	t=0.269, p=0.793	
1 month	48.05±25.06	30.83±9.436
Student	t=-0.643, p=0.535	
3 months	13.14±6.22	9.139±2.51
Student	t=-0.596, p=0.564	
6 months	5.33±3.259	2.11±0.12
Student	t=-0.989, p=0.346	

Note: * The difference between the groups is not statistically significant.

In patients with PVE lesions, statistical significance in the differences between the groups was not obtained.

4. Conclusion

As a result of evaluating the results of laboratory studies, it can be concluded that the inclusion of an ozone-air mixture in the complex treatment of endodonto-periodontal lesions can increase the effectiveness of treatment of patients with this pathology.

Application of artificial intelligence: The review is written without the use of artificial intelligence technologies.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

Mechanisms of the Antistress Effect of Thymus Peptides

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Abstract: The review presents the results of research conducted from 2008 to 2020 at the Department of Pharmacology of the Pediatric Faculty of the GBOU VPO RNIMU of the Ministry of Health of the Russian Federation, in the Laboratory of the Evolution of Memory Mechanisms of the Department of Higher Nervous Activity of the Faculty of Biology of the Lomonosov Moscow State University and in the Laboratory of Molecular Immunology of the Federal State Budgetary Scientific Institution "Research Institute of Physico-Chemical Medicine" of the FMBA of the Russian Federation. To understand the processes underlying the antistress effect of thymus peptide preparations (thymulin, tactivin, and the 5th fraction of thymosin), radioligand binding to the GABAA receptor, neurochemical studies, and experiments using non-selective opiate and serotonin receptor blockers have been conducted. Based on the data obtained, it is assumed that by triggering the cytokine cascade, thymus peptides increase the level of the inhibitory amino acids taurine and glycine, alter the balance of monoamines serotonin/norepinephrine in favor of the former and activate opioid neurons, which in turn limits the damaging effects of stress reactions.

Keywords: stress, stress-limiting system, functional disorders, thymus, thymalin, tactivin, 5th fraction of thymosin.

Citation: Kiseleva N. Mechanisms of the Antistress Effect of Thymus Peptides. Journal of Clinical Physiology and Pathology (JISCPP) 2025; 3 (2): 41-47.

https://doi.org/JISCPP.2024-3-2.41-47

Academic Editor: Igor Kastyro

Received: 24.04.24
Revised: 11.05.24
Accepted: 27.05.24
Published: 28.06.24

Publisher's Note: International Society for Clinical Physiology and Pathology (ISCPP) stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

The problems of stress as the most common nonspecific response of the body to any stimulus and adaptation to it concern not only physiologists, but also doctors. Back in 1936, H. Selye, having put forward the theory of adaptation syndrome, showed that in any stressful state a three-phase process occurs in the body, in which the main shifts are detected by the endocrine organs, mainly the pituitary gland, adrenal glands and thymus. However, in modern schemes of stress and stress-limiting systems, neither the thymus nor the peptides produced by it found a place [1]. It is assigned only the role of a stress-dependent organ.

At the same time, there are close direct and inverse connections between the thymus and the hypothalamic-pituitary system. It is known that shutting down the function of the pituitary gland leads to aplasia of the thymus, a decrease in the secretion of its hormones by epithelial cells and an acceleration of age-related gland involution [2]. Direct regulation of thymus activity by the hypothalamus can be carried out through the sympathetic and parasympathetic parts of the autonomic nervous system [3; 4]. In turn, the neuroendocrine centers of the hypothalamus differentiate during embryogenesis under the influence of the thymus [5]. Various fractions of thymosin and opioid peptides secreted by the thymus stimulate the synthesis of certain releasing factors, followed by increased secretion of gonadotropins and prolactin [6]. At the same time, there is evidence in the literature that various peptide preparations of the thymus affect not only the activity of the immune system, but also affect all types of metabolism [7-13]. At the same time, their action is diametrically opposed to the action of GC, which are one of the leading participants in the implementation of the stress system [1].

In addition, there are known facts of correction of various types of stress. Thus, under infectious stress, the introduction of tactivin (a preparation of thymus polypeptides) into the body of children suffering from acute respiratory diseases led to a rapid recovery of immune status indicators and recovery of sick children [14-17]. Its inclusion in the complex of therapy for visceral leishmaniasis led to a significant reduction in both the duration of treatment for children [18] and leveled the toxic effect of the 5-valent antimony drug pentostama (Great Britain). To correct intoxication stress, tactivin was administered to Wistar rats, which contributed to the effect of systemic

detoxification in total endotoxemia in experimental acute peritonitis caused by gram-negative microflora [19], and in chronic benzene intoxication in male CBA mice, a decrease in the activity of cytochromes P-450, C-reductase, benzpyrenhydroxylase, and epoxide hydrase was noted.

Tactivin monotherapy led to normalization of enzyme activity. At the same time, with subacute intoxication, accompanied by increased activity of enzymes of the second phase of xenobiotic metabolism: glutathione-S-transferase and epoxidihydraz, the drug reduced their activity [20; 21]. An equally pronounced effect of the drug was obtained in rats with a burn injury accompanied by a violation of the function of vital organs. The administration of tactivin eliminated signs of intoxication, reduced the zone of secondary necrosis, and promoted the formation of full-fledged skin regenerates with the formation of hair follicles and sebaceous glands [22]. A synthetic pentapeptide similar to a fragment of thymopoietin (from the 32nd to the 36th amino acid residue), thymopentin has a protective effect in case of stress damage to the stomach [23]. When intoxicated with heavy metals (in particular, lead poisoning), oxidative stress develops, in which pronounced cognitive disorders are observed, which were also eliminated by the administration of tactivin [24].

However, in the examples listed above, the immune system exerted too much influence on the course of the process, and therefore it was difficult to separate the direct immunomodulatory and antistress effects of the thymus drug. To confirm the assumption about the involvement of the thymus in the work of the stress-limiting system, it was necessary to use models of stress-related effects in which the involvement of the immune system would be less significant. To do this, in joint work conducted from 2008 to 2020 at the Department of Pharmacology of the Pediatric Faculty of the state budgetary educational institution of higher professional education RNIMU of the Ministry of Health of the Russian Federation, in the Laboratory of the Evolution of memory Mechanisms of the Department of Higher Nervous Activity of the Faculty of Biology of the Moscow State University. Experimental studies have been conducted in the Laboratory of Molecular Immunology of the Federal State Budgetary Institution "Research Institute of Physico-Chemical Medicine" of the FMBA of the Russian Federation, which have proved that thymus peptides have anxiolytic activity [25-27] and antistress activity under emotional stress, both during learning [24; 28-30] and against the background of functional disorders avoidance reactions (failure of the conditioned reflex of active avoidance and a change in the location of the hole) [31-35].

The purpose of this work was to summarize the data obtained on the mechanisms of the effect of thymus peptides (tactivin and thymosin fractions 5 and thymulin) on the functioning of the stress-limiting system.

2. Materials and Methods

The experiments were conducted on Wistar rats weighing 180-200 g. The animals were kept in standard vivarium conditions with free access to water and food and a 12-hour light-dark regime. Before starting the study, the level of motor activity of animals in a mink chamber and an open field was tested in all animals to form groups of identical behavior. For 5 days (at the same time), once a day, experimental animals were intraperitoneally injected with a preparation of thymus polypeptides (tactivin, the 5th fraction of thymosin or thymulin), reference (piracetam, diazepam) and control drugs (saline solution, spleen polypeptides prepared in a similar way to tactivin and/or solvent for timulin) in a volume of 0.5 ml. All experiments were conducted in accordance with the Principles of Good Laboratory Practice (National Standard of the Russian Federation GOST R 53434-2009) and the provisions of the International Convention on "Rules for Working with Experimental Animals" (European Communities Council Directives, November 24, 1986, 86/609/EEC).

Radioligand analysis of GABAA receptors (the experiments were conducted jointly with the Laboratory of Radioisotope Research Methods of the V.V. Zakusov Research Institute of Pharmacology of the Russian Academy of Medical Sciences, head, Doctor of Medicine, Professor G.I. Kovalev). For the ex vivo experiment, tactivin was administered intraperitoneally for 5 days once a day at a dose of 0.5 mg/ kg (experimental group, n=10), or 0.5 ml of saline solution (control group, n=10). The animals were removed from the experiment after 24 hours after the last injection. After decapitation, the hypothalamus and frontal cortex were isolated from the brain, which were immediately frozen in liquid nitrogen and stored in a low-temperature refrigerator at - 85 °C. The preparation of membrane preparations containing GABAA receptors in these structures was carried out using modified methods [36; 37]. The protein concentration in the samples was determined using the standard Lowry O.N. method [38].

Biochemical determination of the content of monoamines, excitatory and inhibitory amino acids in the rat brain (part of the experiments were conducted jointly with the Laboratory of Neurochemical Pharmacology of the V.V. Zakusov Research Institute of Pharmacology of the Russian Academy of Medical Sciences, head, Candidate of Medical Sciences, V.S. Kudrin). Prior to the start of the conditioned response of active avoidance production, experimental groups of animals received one of the studied thymus peptide preparations (timulin, tactivin, or the 5th fraction of

thymosin) for 5 days, and control animals were injected with saline/spleen peptides/ thymulin solvent. When the number of avoidance reactions reached 80%, a functional violation of the conditioned response of active avoidance (failure of the avoidance reaction) was performed. The animals were decapitated 1 hour after the last injection of the drugs. The brain was extracted on ice and the frontal cortex, hypothalamus, nucleus accumbens, striatum, and hippocampus were isolated. The structures were frozen and stored in liquid nitrogen. On the day of the study, the structures were homogenized, and a supernatant was prepared in which monoamines and metabolites, or amino acid content were determined by high-performance liquid chromatography with electrochemical detection [39].

Statistical processing of research materials.

Statistical processing of the obtained data was carried out using the STATISTICA 8.0 program (Statsoft, USA), using the Student's t-test, nonparametric criterion, Mann-Whitney, as well as using Fisher's F-test for radioligand analysis.

3. Results

Activation of the GABAergic system

Using ex vivo radioligand analysis, no statistically significant differences were found in the number of GABA receptors in control and experimental animals not exposed to emotional stress. The results of the ex vivo experiments were evaluated using Cm and B-Max values, reflecting the degree of affinity of the receptor to the ligand (nM) and the number of ligand binding sites (fmol/mg protein), respectively. The results did not reveal significant differences in the number of receptors studied in the hypothalamus (Fig. 1) and in the frontal cortex between the control and experimental groups.

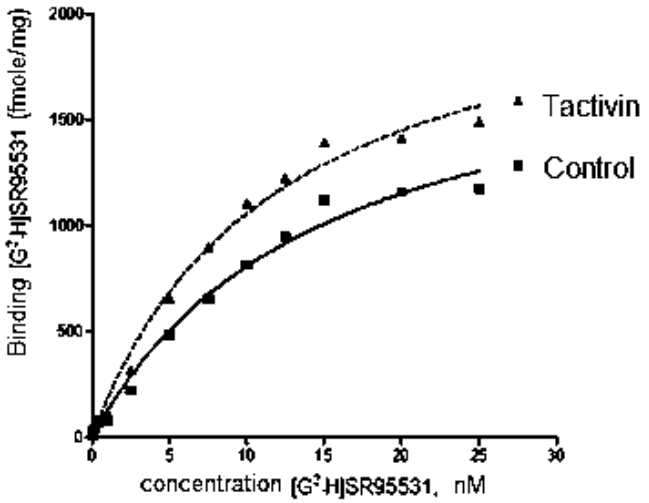

Figure 1. Binding characteristics of the selective ligand of the GABAA receptor [G-3H]SR95531 to the hypothalamic membranes of Wistar rats (intact animals) (n=20). Fischer's F-test, p=0.30

GABA levels in some structures of the rat brain were studied using high-performance liquid chromatography with electrochemical detection against the background of emotional stress caused by a malfunction of the avoidance reaction. As can be seen from the data presented in Table 1, the administration of tactivin to animals exposed to emotional stress caused by a malfunction of the avoidance reaction did not affect the level of the inhibitory amino acid GABA in the studied structures of the rat brain [40].

Table 1. GABA level (mmol/d) in the brain structures of Wistar rats on the background of emotional stress (failure of the conditioned reaction of active avoidance) (n=20)

Composition	EVPGABA content in brain structures in animal groups		The level of significance of the difference in results (p control, - experience)
	control	against the background of tactivin	
The Hypothalamus	0,844 ± 0,057	0,813 ± 0,056	0,7
The Frontal cortex	0,415 ± 0,009	0,406 ± 0,004	0,423

The nucleus accumbens	1,032 ± 0,059	0,988 ± 0,048	0,59
The Striatum	0,549 ± 0,017	0,538 ± 0,026	0,747
The Hippocampus	0,451 ± 0,017	0,442 ± 0,019	0,725

Thus, the stress-protective activity of the thymus polypeptide drug tactivin under emotional stress does not seem to be associated with the activation of the GABAergic system [41].

The level of monoamines in the structures of the brain

High-performance liquid chromatography with electrochemical detection was used to study the level of monoamines in some brain structures against the background of thymus peptides. The administration of tactivin [42], as well as thymulin and thymosin fraction 5 [43], significantly reduced dopamine levels in intact rats in the frontal cortex. The level of dopamine in the striatum increased statistically significantly, which explains the increase in motor activity against the background of the drug in behavioral tests. An increase in norepinephrine levels was also noted in the hippocampus.

After emotional stress caused by a malfunction of the avoidance reaction, on the background of tactivin [42], there was a statistically significant increase in serotonin and norepinephrine in the frontal cortex, hypothalamus and striatum (Fig. 2). Similar changes were observed with the use of thymulin and thymosin fraction 5 [43].

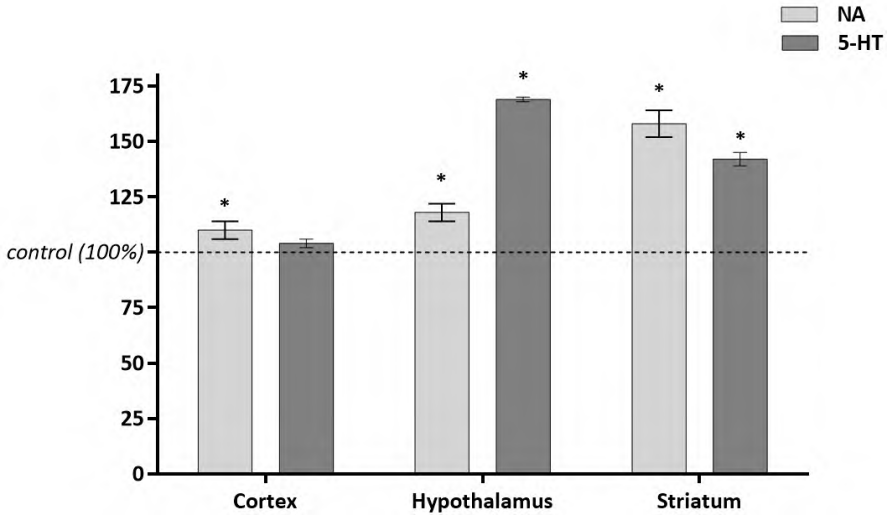

Figure 2. Changes in the level of monoamines in the rat brain on the background of tactivin (failure of the avoidance reaction)
Note: the indicators of the control group are taken as 100%. NA – norepinephrine; 5-HT – 5-hydroxytryptophan (serotonin)

Since these changes were not observed in intact rats, these changes in the monoamine content can be explained by the development of emotional stress in animals caused by a functional violation of the avoidance reaction. Apparently, thymus peptide preparations alter the serotonin/norepinephrine ratio in favor of the former. Considering that an increase in serotonin content is a compensatory response to stress, and a decrease in norepinephrine concentration is associated with manifestations of fear [44; 45], it can be assumed that such a change underlies the positive effect of thymus peptides upon failure of the conditioned active avoidance reaction.

4. Discussion

The level of excitatory and inhibitory amino acids in rat brain structures

When studying the levels of excitatory and inhibitory amino acids in rats without emotional stress, it was found that tactivin (0.5 mg/kg) causes a statistically significant increase in the content of taurine in the hypothalamus and glycine in the frontal cortex [40].

An increase in glycine content in the frontal cortex is observed in selank, which explains its anxiolytic effect associated with the aminoacidergic system of the brain [46]. The prefrontal cortex is responsible for the implementation and coordination of higher cognitive processes in the planning of complex behavioral acts [47]. The frontal cortex is involved in decision-making based on emotional reactions and thus provides the motor component of the emotional response. An increase in glycine levels may reflect the neuroprotective properties of tactivin, which are manifested in the prevention or reduction of manifestations of emotional stress in rats [25; 31-35].

The hypothalamus belongs to the mesolimbic dopaminergic system, which is largely responsible for the development of anxiety states [48]. The administration of tactivin at a dose of 0.5 mg / kg significantly increased the taurine content in the hypothalamus by 1.3 times. Taurine is involved in the regulation of hormone secretion, acetylcholine and GABA [49]. The mechanism of action of taurine includes binding to GABAA receptors, as well as to certain types of glycine-binding receptors, which in turn stimulates the release of vasopressin and oxytocin [50].

Based on these data, it is possible to suggest a possible mechanism of action of thymus peptides on the central nervous system through cytokine cascades. It is well known that thymus peptides activate the T-cell link of immunity, while increasing the production of various cytokines: IL-1, IL-2, IL-6, IL-8, TNFa, and IFu [1; 51; 52]. IL-1 increases the level of taurine in the hypothalamus [53], which is consistent with the neurochemical data presented by us. By increasing the production of IL-1, thymus peptides increase the level of norepinephrine in the hippocampus [32]. Thus, on the one hand, they normalize immunological parameters in case of deviation from the norm in one direction or another, without causing critical disorders [14; 54], and on the other hand, they increase the level of inhibitory amino acids in structures associated with emotional response [40].

The opioid system

Since electrical pain irritation is the main stressful factor in the formation of functional disorders of URPI, it is logical to assume that thymus polypeptides have analgesic activity. It is known that two antagonistic systems are involved in pain regulation: nociceptive and antinociceptive. The main mediators of the antinociceptive system are opioid peptides and serotonin. Thus, the most likely mechanisms of substances with analgesic activity are associated with the activation of the serotonergic and/or opiate systems. Studies have been conducted to study the analgesic activity of thymus peptides using a non-selective opioid system blocker (naloxone (5-alpha)-4,5-epoxy-3,14-dihydroxy-17-(2-propenyl)morphinan-6-ONE (in the form of hydrochloride, 0.4 mg/1 ml, manufactured in Poland, administered intraperitoneally at a dose of 1 mg / kg) and a non-selective serotonin receptor blocker (cyproheptadine, manufactured by Sigma, administered intraperitoneally at a dose of 1 mg / kg, 10 minutes before naloxone injection). The "tail twitching" test was used to assess the pain sensitivity of the animals. In all animals, the background value of the latent period (LP) of tail twitching was measured, in relation to which further interpretation of the results was performed. Administration of thymus peptides for 5 days increased the LP of tail twitching, while administration of naloxone 20 minutes before testing reduced it to background values [55-61], thus, administration of thymus peptides leads to an increase in the pain threshold due to activation of the opioid system

Stress-induced analgesia caused by various stressful effects (PCL, Porsolt test, emotional stress caused by a malfunction of the avoidance reaction, acute and chronic immobilization stress) led to an increase in the threshold of pain sensitivity, that is, led to the development of stress-induced analgesia [62; 55; 56; 61]. The administration of naloxone against the background of stress-induced analgesia led to a decrease in tail twitching to baseline values. Thus, the nature of stress-induced analgesia in control animals is determined by the activation of the opioid system alone [56]. An increase in the threshold of pain sensitivity after stress was also observed against the background of thymus peptides, but to a lesser extent than in the control. Thus, the opiate receptor blocker in these animals only partially eliminated the effect of analgesia. The sequential administration of ciproheptadine and naloxone led to a sharp decrease in the pain threshold, which turned out to be lower than the background value, but exceeded the control value [56], that is, this type of stress analgesia, which developed against the background of thymus peptides, has a mixed form of stress-induced analgesia containing opioid and non-opioid (seratonergic) components.

5. Conclusion

It is known that any stressor activates stress and stress-limiting systems, primarily by increasing the activity of the GABA and opioid systems, which leads to a limited stress response. At the same time, an increase in serotonin levels in brain structures is considered as an adaptation option under stress. Shifts in the balance towards serotonin against the background of thymus peptides under stress and activation of the opiate system underlie their stress-protective effect.

Apparently, activation of the stress system simultaneously activates the thymus and increases the level of its polypeptides, which are functional antagonists of the stress system. They increase the body's resistance to stress. The thymus is not only the central organ of the immune system, but also the organ of the stress-limiting system.

Taking these facts into account, the work of the stress-limiting system can be represented as follows. Activation of the stress system leads to stimulation of the thymus through the sympathetic and parasympathetic parts of the autonomic nervous system. This leads to an

increase in the level of thymic peptides, which trigger a cytokine cascade with an increase in the production of various cytokines (IL-1, IL-2, IL-6, IL-8, TNFa, and IFc). In turn, cytokines increase the level of the inhibitory amino acids taurine and glycine, alter the balance of monoamines serotonin/norepinephrine in favor of the former, and activate opioid neurons, which limits the damaging effects of stress reactions.

Thus, under stress, not only the hypothalamic-pituitary-adrenal axis is activated, but also the hypothalamic-pituitary-thymus system, which limits the stress-damaging effect of excessive activity of the hypothalamic-pituitary-adrenal axis.

Application of artificial intelligence: The review is written without the use of artificial intelligence technologies.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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