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Cell-free DNA in the Cerebrospinal Fluid under Emotional Stress

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ABSTRACT

Background: Cell free circulating DNA (cfDNA) in blood is known to be a tumor marker however there is no information about its concentration in cerebrospinal fluid (CSF) in control and in emotional stress (ES). The aim of the study was to determine level of cfDNA in CSF of rats with different resistance to stress before and after ES. **Methods:** A total of 19 male Wistar rats weighing 200-220 g were included in this study. All rats were divided into 2 groups depending on the motor activity: active (prognostically resistant to ES) – 9, animals predisposed to ES (passive) – 10. CSF samples were collected twice by puncture of the cisterna magna of the brain before and after stress in the interval of 10 days. CfDNA was detected by phenol method. Statistical data analysis was performed with SPSS 16 for Windows. **Results:** Tendency to higher level of cfDNA in active rats compared with other groups has been found in our experiments. All animals statistically significant were divided into two groups: with high and low concentration of cfDNA in CSF. 70% animals with high level of cfDNA in CSF were active rats and 30% - passive. After stress the concentration of cfDNA in active animals demonstrated a tendency to decrease, while the passive - on the contrary, to increase after ES. Furthermore, the inverse correlation between the change of cfDNA and volume of CSF aliquot was identified. **Conclusion:** We have proved the presence of cfDNA in rats` CSF. cfDNA concentrations differ in active and passive rats. It`s level decreases in active rats and increases in passive after ES. The higher concentration of cfDNA in active rats possibly related to the low activity of endonuclease compared with passive animals. This data can be compared with similar data from earlier studies of cfDNA in serum.Volume of CSF changes in active and passive rats differently: in active, it increases - in passive decreases after ES. We cannot explain this phenomenon. Therefore, to clarify these issues and to determine the source of cfDNA in CSF further studies are needed.

KEYWORDS

Stress Resistance, Rat, Open Field, Motor Activity Index

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INTRODUCTION

The cell-free DNA (cfDNA) circulating in blood was studied in patients with different pathologic processes [2,4,5,6,11,14,15,16].

Yet it is unclear if cfDNA had an impact on emotional stress development and stress resistance. Although there was study demonstrated an increase of cfDNA concentration in the rats' blood after stress.[8,9]

The hypothesis was that cfDNA released after emotional stress (ES) in brain tissue could be detected in cerebrospinal fluid (CSF) samples. There were a lot of studies in which cfDNA was detected in CSF in different pathological processes.

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Rhodes et al. (1995) found the pathological cfDNA in the cerebrospinal fluid of patients with neoplasms on the nervous system. Another report proves existence of cfDNA in the CSF of patients with Parkinson disease.[7] The fetal cfDNA was found in the CSF of pregnant women during the peripartum period. [1,12] However, reports about cfDNA in CSF after ES wasn`t found.

The aim of the present study is to detect cfDNA concentration in the CSF of rats before and after ES and compare results of two groups of animals with different emotional stability (stress resistance).

METHODS

Study was performed in the I.M.Sechenov First Moscow State Medical University (MSMU) and Research Centre for Medical Genetics of the Russian Academy of Medical Sciences (RCMG) in 2013.

A total of 19 male Wistar rats weighing 200-220 g were tested during this study. The animals underwent routine cage treatment once a week and microbiological monitoring in accordance with the rules of the Federation of European Laboratory Animal Science Associations. Food and water were available *ad libitum*. Animal care procedures were performed in line with international guidelines of laboratory animals treatment.

All the rats were tested in an open field and divided into two groups in accordance with the Motor Activity Index (MAI). MAI was calculated as a ratio of the sum of crossed peripheral and central sectors of open field and the sum of latent periods of the first movement and entrance to the arena center. As demonstrated earlier, animals with MAI > 1.5 showed high stress resistance compared to rats with MAI < 0.8. [10] 9 active rats (prognostically resistant to ES) were referred to have MAI more than 1.5 and 10 passive rats (prognostically predisposed to ES) had MAI less than 0,8.

For the purpose of the CSF sampling all rats were anesthetized with chloralhydate (0.45 mg per 100g). After anesthesia the CSF was collected by puncture of the cisterna magna of the brain.[13] The CSF collection was carried out within 3 minutes. There was an interval of 10 days between two CSF collections for each rat. The second CSF sampling was carried out after ES: immobilization on a flat platform by fixation of four limbs for 2.5 hours. The CSF samples were immediately frozen and stored at -20^{0}C.

CfDNA was detected by phenol method in the samples diluted by saline to 400µl. [3,7,19] CfDNA concentration was measured by Picogreen fluorescence (Invitrogen, USA) on fluorimeter Enspire™ 2300 (Perkin Elmer) at excitation wavelength - 480 nm, emission - 520 nm.

Statistical data analysis was performed with SPSS 16 for Windows (SPSS Inc., Chicago, USA). The hypothesis on the difference of independent groups was proved by the nonparametric Mann-Whitney U-test. Experiment results were presented as median weight with interquartile range (Me) [25%; 75%]. The Spearman's correlation coefficient was used to analyze the connection of features.

RESULTS

Experimental animals were divided into two groups ($p < 0.005$, U-test) with statistically significant difference in the CSF cfDNA concentration – with high 6.5 ng/ml [6.2, 7] (n = 9) and low 2.1 ng/ml [2; 2.1] (n = 13) cfDNA concentration per probe. The median concentration of cfDNA in CSF the test animals made 27 ng/ml in passive and 68 ng/ml in active rats (Table 1). Although the difference between active and passive rats was not statistically significant, active rats from the test group demonstrated a predisposition to a higher cfDNA level in comparison to passive ones (Fig.1). The correlation of rats with the low CSF cfDNA concentration among active and passive animals made 32% (6 animals) and 68% (13 animals) respectively. The same balance was also observed after ES. The volume of the CSF collected in our experiments was approximately the same for all groups of animals.

Median concentrations of cfDNA in the CSF of rats after ES made 32 and 46 ng/ml for passive and active animals respectively, so the difference of cfDNA concentration between active and passive rats was not significant. According to the assessment of the corresponding upper limits of the interquartile range, the cfDNA concentration in the CSF after ES showed a tendency to decrease in active animals and to increase in passive rats.

The inverse correlation was found between cfDNA concentration and the CSF volume in both groups of animals. Spearman correlation coefficient (R) made -0.73 for passive rats (n = 10) and -0.78 for active ones (n = 9) ($p < 0.05$).

After ES some changes in cfDNA concentration were observed in 5 of 9 active animals: in 4 out of these 5 animals a decrease of the cfDNA concentration was accompanied by an increase in liquor aliquot volume, in the 5th active rat cfDNA concentration was growing but the volume of the CSF aliquot was reducing (Fig 2). Changes of cfDNA concentration were detected after ES in 6 of 10 passive animals: in 4 of them an increase of cfDNA concentration was accompanied by a decrease of the CSF volume, in 2 rats of these 6 a reduction of the cfDNA concentration was observed – in the first rat the CSF volume increased after stress, and in the second one the CSF volume was not changed.

The cfDNA level in active rats was not changed after ES in 8 of 9 animals (Fig 1). In one rat a decrease in the cfDNA concentration after stress was observed. No change of the cfDNA concentration was found in 8 out of 10 passive animals, and 2 rats faced an increase and a decrease of the cfDNA concentration. (Fig 1).

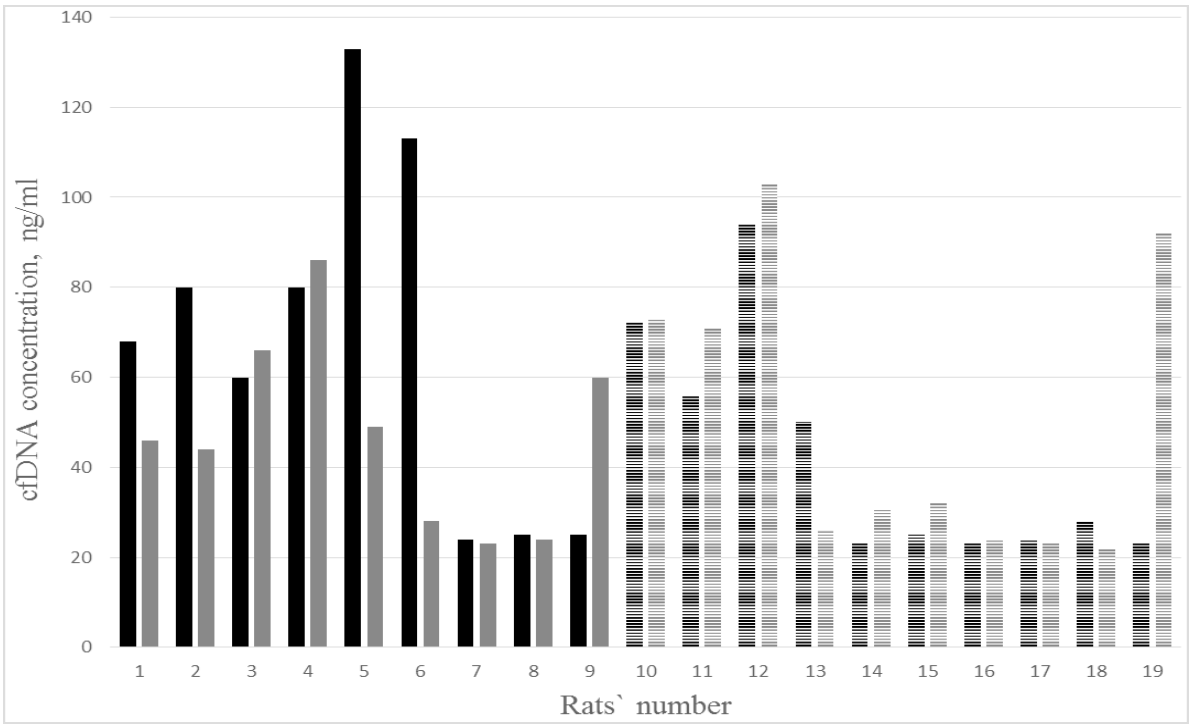

Fig 1. CfDNA concentration in the CSF of rats before and after stress in active and passive rats. Active group: – before stress, – after stress. Passive group: - before stress, - after stress. The bars represent the concentration of cfDNA in the CSF of each rat. The difference between the active and passive groups was not statistically significant (p= 0.5)

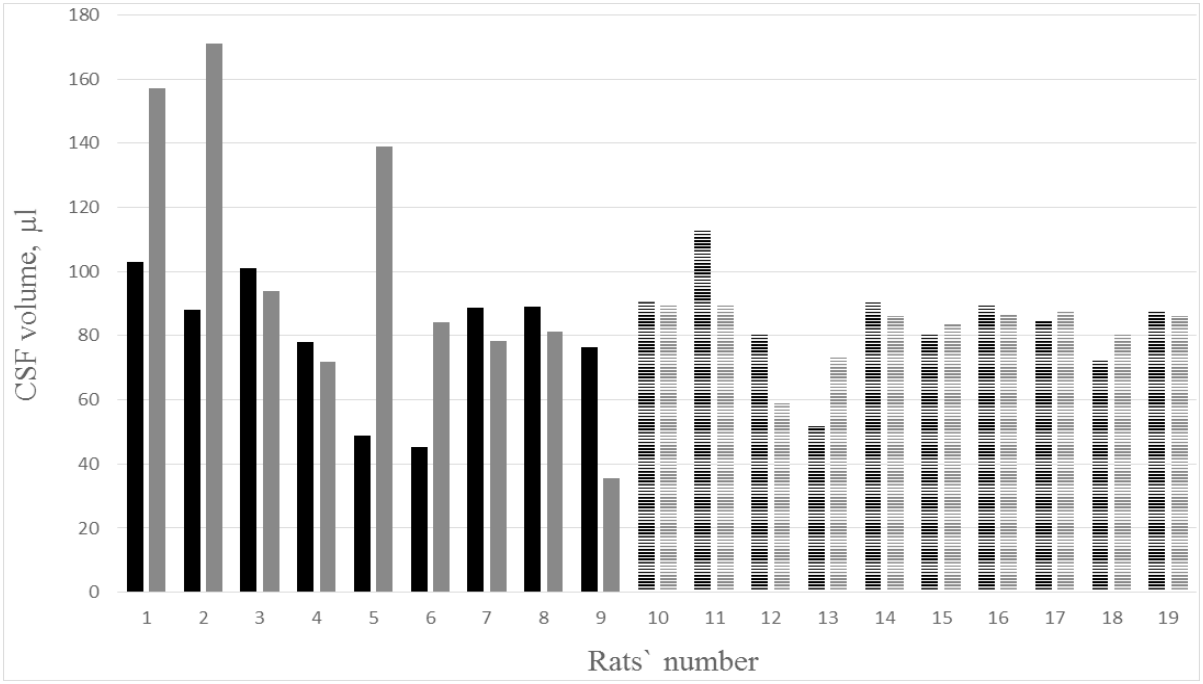

Fig 2. The CSF aliquot volume in active and passive rats before and after stress. Active group: – before stress, – after stress. Passive group: - before stress, - after stress. The bars represent the CSF volume of each rat.

	Groups of animals			
	passive rats n=10		active rats n=9	
	before ES	after ES	before ES	after ES
cfDNA concentration, ng/ml	27 [23;56]	32 [24;73]	68 [25;80]	46 [28;60]
CSF volume, µl	86 [81;90]	86 [81;87]	88 [76;89]	84 [78;139]
Total amount of cfDNA, ng	2.1 [2.0;6.3]	2.7 [2.0;6.4]	6.1 [2.2;6.5]	6.2 [2.1;6.8]

Table 1. The CfDNA and CSF volume in the cisterna magna of active and passive rats before and after emotional stress

DISCUSSION AND CONCLUSION

This research is devoted to the study of cfDNA concentration in the CSF from the cisterna magna of animals with different prognostic resistance to ES. The results of our research coincide with the data on the cfDNA level in the blood plasma of rats with different emotional stability: active animals had higher concentration of cfDNA than passive ones.[9] The difference of cfDNA concentration in blood was more visible than in the CSF. The cfDNA concentration in the blood plasma made 60 [52; 69] (n=17) ng/ml in active rats and 155 [134; 174] (n=11) ng/ml in passive ones respectively. Consequently, cfDNA concentration in the CSF is 2.1-3.0 times lower than in the blood plasma of active and passive animals. The same cfDNA blood/CSF distribution was found in patients with Parkinson disease: cfDNA concentration in the plasma was 3.3 times higher than in the CSF.[7]

The possible sources of cfDNA are not quite clear and may include necrotic and apoptotic cells. Also cfDNA may be excreted by active secretion.[18] Excitotoxicity and inflammation, as well as ES, which is inducing oxidative stress (OS), may result in the massive death of cells and the cfDNA emission to the extracellular space and blood.[9,20] Stroun and Anker (1972) proved the ability of cfDNA to penetrate through the blood-brain barrier. Chronic diseases like Parkinson`s disease, Alzheimer disease or, malignant neoplasms induce visible changes in cfDNA concentration in the CSF.[7,16,17] Acute ES effects may not cause such significant changes since cfDNA released to the extracellular space after the death of cells may be absorbed by neighboring cells or destroyed by the extracellular endonucleases before reaching ventricles.

All experimental animals were divided into the groups with significantly high and low total amount of cfDNA in the CSF. Animals with the high cfDNA level prevail in-a group of active rats, and rats with the low level of cfDNA in the CSF, on the contrary, prevail in a group of passive ones. Therefore, our study suggests that cfDNA may serve as an indicator of emotional stability and stress reactivity of rats. The inverse correlation between the CSF volume and cfDNA concentration in the CSF was found. The total cfDNA level remained mainly constant before and after ES.

Conclusion

Some differences in cfDNA concentration in the CSF of active and passive rats were found. Active (stress resistant) rats demonstrated a predisposition to a higher cfDNA level in the CSF in comparison to passive (stress-predisposed) animals. After ES the cfDNA level decreases in active rats and increases in passive ones. The CSF volume after ES of active and passive rats changes in a different way: it increases in active rats and decreases in passive ones. We need to continue our studies to explain these effects and determine the source of cfDNA in the CSF. Therefore, our data suggests that the CSF cfDNA may be used as a stress indicator.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

AUTHOR CONTRIBUTION

All authors contributed to the study design, interpretation of the literature data, and the manuscript drafting. All authors read and approved the final version of the manuscript for publication.

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