The elemental analysis of brain tissue in case of pilocarpine induced epilepsy and the treatment of tacrolimus (FK-506), a neuroprotective agent

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Although, the epilepsy has been a serious problem of clinical neurology for many years, its pathogenesis in many cases has still been unknown. The epileptic seizures induce neurodegenerative changes in brain and particular vulnerability to them occurs for the areas such as the hippocampal formation, cortex and amygdaloid nuclei [1]. In the last years, it has been searched for the pharmacological methods that could decrease the effects of brain injury. The substances increasing the survival of nerve cells in the pathologically changed tissue and simultaneously promoting the regeneration of damaged axons and decreasing the inflammation have been found. One of them is FK-506, an immunosuppressive drug used mainly after allogenic organ transplantation in order to reduce the activity of the immune system and so the risk of organ rejection [2].

The following project is the continuation of our investigation concerning the role of trace elements in the pathogenesis and progress of pharmacologically induced epilepsy. In frame of it, the influence of neuroprotective agent FK-506 on the elemental composition of brain tissue in case of rats with pilocarpine-induced epilepsy was analyzed. The obtained results were compared with ones obtained previously for the groups of epileptic and naive control animals.

Unfixed and non-embedded rat brains were cut frontally using a cryomicrotome into 15 μm thick slices. The sections containing the dorsal part of the hippocampus and the cerebral cortex were mounted on the Ultralene foil and freeze-dried.

The topographic and quantitative elemental analysis of the tissues was done using X-ray fluorescence microscopy. The measurements were carried out at HASYLAB beamline L. The multilayer monochromator was applied and the primary photon energy was set to 17 keV. The polycapillary optics was used for the focusing of the beam and the obtained beam spot had the dimension of 15 μm x 15 μm. The X-ray fluorescence spectra were measured using the Vortex SDD detector from SII Nano Technology USA inc. and the time of single spectrum acquisition was equal to 6 s. Measurements of NIST standard reference materials (SRM 1833 and SRM 1832) were performed for spectrometer calibration.

The elements such as P, S, Cl, K, Ca, Fe, Cu, Zn, Br, Rb and Sr were detected in rat brain sections. For each sample, the two-dimensional analysis of elemental distribution was performed for the areas of hippocampus and brain cortex. The results of such analysis for the hippocampus from selected FK-506 – treated epileptic (SNF) rat and untreated epileptic (SNS) animal were shown in Figure 1.

For five selected brain areas, i.e. CA1 and CA3 regions of Ammon's horn, DG – dentate gyrus and H – hilus of dentate gyrus and parietal cortex the mean masses per unit area were evaluated. The areas taken into account in calculations were equal to 300 μm per 300 μm. In order to compare the elemental composition of FK-506 – treated epileptic group (SNF) with untreated epileptic (SNS) and control (CS) animals the median values of masses per unit area were evaluated for all the analyzed brain areas in the three examined groups. The statistical significance of differences between medians was tested with non-parametric U (Mann-Whitney) test. The results of statistical analysis were presented in the Table 1.

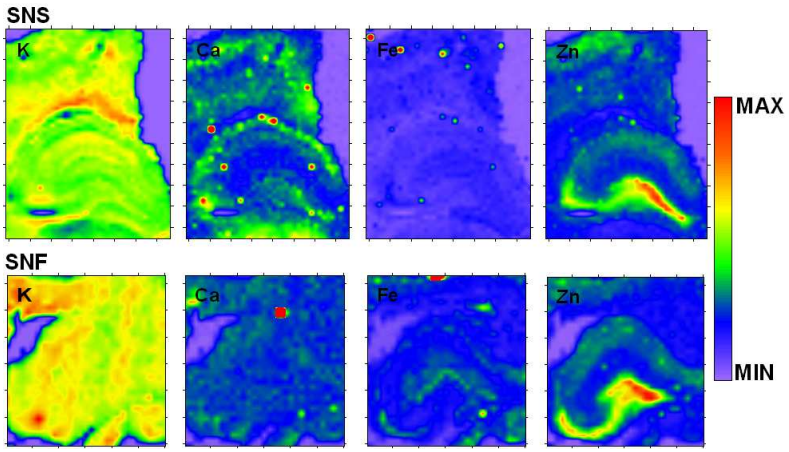

Figure 1. The maps of elemental distribution obtained for selected FK-506 – treated epileptic (SNF) rat and untreated epileptic (SNS) animal.

Table 1. The p-values of U (Mann-Whitney) statistics.

	Elements(*)							
	P		**Cl**	**K**	**Ca**	**Fe**	**Cu**	**Zn**
Area	**SNF vs SNS**							
CA1	**0.09**	0.20	0.67	**0.09**	0.39	0.52	0.29	0.52
CA3	**0.09**	**0.09**	0.39	**0.02**	0.39	**0.09**	0.67	**0.09**
DG	0.20	0.39	0.83	**0.09**	0.67	**0.06**	0.29	0.14
H	0.20	0.14	0.29	**0.03**	0.83	0.29	1.00	0.20
C	0.20	**0.09**	0.39	**0.06**	0.52	0.20	**0.03**	**0.09**
Area	**SNF vs CS**							
CA1	0.14	0.14	0.22	0.14	0.46	0.46	0.22	1.00
CA3	0.46	0.62	0.22	0.14	0.81	0.46	1.00	0.81
DG	0.81	0.81	0.81	0.62	0.81	1.00	0.62	0.62
H	0.62	0.62	0.33	0.22	0.81	0.62	0.62	0.62
C	0.14	**0.09**	0.62	0.33	0.46	0.62	0.33	0.33

(*) The elements for which the mean masses per unit area were lower than the detection limits were omitted from the table.

As one can notice from the Table 1, many statistically significant differences in elemental composition was observed between SNF and SNS animals but only one between SNF and CS group.

Acknowledgements:

This work was supported by the Polish Ministry of Science and Higher Education, DESY and the European Community (Contract RII3-CT-2004-506008 (IA-SFS)).

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