

Apoptosis and autophagy, as inherent components of autoimmunity in the acute period of ischemic stroke

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Acute ischemic stroke is accompanied by aseptic inflammation, which alters the brain tissue and exposes the co-stimulatory molecules of the immune system and the neuronal antigens. It is known that apoptosis, autophagy, and necrosis are the main mechanisms of neuronal death after acute ischemic stroke. Modulation of apoptosis and autophagy in the acute period of the disease, as components of increased immunological reactivity, can contribute to the survival of neurons, preventing their delayed death. A comparative assessment of the dynamics of apoptosis and autophagy markers was performed for better understanding of the cross-interactions between these processes. The results indicate that several markers of apoptosis and autophagy are involved at various stages of the acute period of ischemic stroke and that enhanced spontaneous apoptosis of peripheral blood mononuclear cells acts as one of immunosuppression factors in acute period of the disease. The obtained data confirm the active participation of autophagy, pro- and antiapoptotic processes in the autoimmune response and the formation of delayed neuronal death after acute ischemic stroke.

Keywords: autoimmunity, inflammation, apoptosis, autophagy, acute ischemic stroke, serum level of protein p53, Bcl-2, Beclin 1, LC3.

Introduction

Ischemic stroke (IS) is accompanied by aseptic inflammation, which alters the brain tissue and exposes the co-stimulatory molecules of the immune system and the neuronal antigens [1]. The initial damage to neurons occurs within a few minutes after acute ischemia and is realized by the mechanism of necrosis, apoptosis and autophagy, while autoimmune inflammation, which contributes to the progression of the pathological process, lasts from several days to several months [2]. At the same time, the stroke-induced immune activation may promote reparation phenomena in the brain [1]. According to literature, modulation of apoptosis and autophagy in the acute period of IS, as components of increased immunological reactivity, can contribute to the survival of neurons, preventing their delayed death [3]. Nevertheless, it is not completely clear which of the processes of programmed cell death (PCD) prevails at a particular stage of the ischemic cascade and is more involved in the autoimmune response.

The objective of the study

We proposed that a comparative assessment of the dynamics of apoptosis and autophagy markers will lead to better understanding of the cross-interactions between these processes at different stages of the acute period of IS and evaluate their involvement in autoimmune inflammation.

Materials and methods

All studies were agreed with the University ethical committee. The dynamics of autophagy and apoptosis markers in peripheral blood of 31 patients in the acute period of the newly diagnosed IS in comparison to dynamics of the severity of the neurological condition and the volume of brain damage was studied. Clinical and neurological dynamic examinations with the assessment of neurological deficit using NIHSS (National Institutes of Health Stroke Scale) and determination of the brain infarct volume by MRI scan (Magnetic resonance imaging) were carried out. The intensity of spontaneous apoptosis of peripheral blood mononuclear cells (PBMCs) was evaluated by the number of Annexin V+-cells (Abcam, UK) by flow cytometry (FC 500, Beckman Coulter, USA). The serum levels of p53 apoptosis inducer and Bcl-2 apoptosis inhibitor, autophagy markers Beclin 1 and LC3B were evaluated by ELISA using appropriate test systems (Abcam, UK). The comparison group consisted of 27 healthy donors. Blood sampling was carried out on the 1st, 3rd, 5th, 7th, 9th, 11th, 14th and 21st days after IS. For statistical processing of the obtained data, the nonparametric Wilcoxon-Mann-Whitney test was used.

Results and discussions

Increased level of spontaneous apoptosis of PBMCs was observed already in the first 24 hours after the ischemic attack and persisted for the next 7 days. Statistically significant elevated serum level of p53 was observed during the 9 days (table 1). The increase in p53 protein content positively correlated with the severity of neurological deficit (NIHSS > 10) and the amount of brain damage according to MRI data already on the 1st day after IS ($r=0.79$; $p<0.05$ and $r=0.81$; $p<0.01$ — respectively) and for the next 9 days.

Table 1. The dynamics of the concentration of p53 protein in the blood serum of patients with acute IS (U/ml)

Groups of examined persons	The time elapsed since the development of a stroke						
	1 st day	3 rd day	5 th day	7 th day	9 th day	14 th day	21 st day
	The concentration of p53 (U/ml)						
I	1,2	–	–	–	–	–	–
II	15.9**	26.1***	16.4**	10.8*	8.3*	2.2	1.9

Notes: 1. I — control group (n = 27); II — patients with acute IS (n = 31). 2. * Differences in the studied indicator with the control are statistically significant ($p<0.05$); ** differences in the studied indicator with the control are statistically significant ($p<0.01$); *** differences in the studied indicator with the control are statistically significant ($p<0.001$).

Increased Beclin 1 and LC3 serum levels (table 2) positively correlated with the severity of neurological deficit and the extent of brain damage from the 1st through the 3rd and 1st through the 5th days, respectively, which, in combination with an increase in p53 protein content, probably indicates a joint involvement of apoptosis and autophagy in neuronal death at the early stages of the acute period of IS.

Table 2. The dynamics of the concentration of Beclin 1 and LC3 proteins in the blood serum of patients with acute IS from day 1 to day 5 (ng/l)

Groups of examined	The time elapsed since the development of a stroke					
	1 st day		3 rd day		5 th day	
	Beclin 1 (ng/l)	LC3 (ng/l)	Beclin 1 (ng/l)	LC3 (ng/l)	Beclin 1 (ng/l)	LC3 (ng/l)
I	90.4	102.1	–	–	–	–
II	161.8*	179.8*	172.1*	201.4*	139.4*	158.6*

Notes: 1. I — control group (n=27); II — patients with acute IS (n=31). 2. *Differences in the studied indicator with the control are statistically significant ($p < 0.05$).

A strong direct correlation between the elevated level of Bcl-2 and the large volume of brain damage was observed only from the 11th to the 14th day ($r=0.86$; $p < 0.01$). Perhaps this is due to the time required to activate compensatory antiapoptotic processes.

Conclusions

The data obtained indicate the involvement of p53, Bcl-2, Beclin 1 and LC3 proteins in ischemic brain damage at various stages of the acute period of IS.

The increased level of spontaneous apoptosis of PBMCs is consistent with the literature data that in the acute period of IS there is immunosuppression, which is a predictor of secondary infection and characterized by lymphopenia and monocytopenia [2]. The results obtained indicate that enhanced spontaneous apoptosis of PBMC acts as an additional immunosuppression factor in acute period of IS.

Thus, our data confirm the active participation of autophagy, pro- and antiapoptotic processes in the autoimmune response and the formation of delayed neuronal death after acute IS. However, the question of which of these types of PCD makes a greater contribution to autoimmune inflammation in acute IS remains open and makes the further study of this problem promising.

References

1. Barinov E. F., Yevtushenko S. K., Maksimenko T. L., Barinova M. E., Tverdokhlebov T. A., Yevtushenko I. S. Mechanisms of regulation of the inflammation in ischaemic brain. *Mezhdunarodnyi nevrologicheskii zhurnal*, 2013, vol. 8, no. 62, pp. 13–21. (In Russian)

2. Tsygan N. V., Trashkov A. P., Litvinenko I. V., Yakovleva V. A., Ryabtsev A. V., Vasiliev A. G., Churilov L. P. Autoimmunity in acute ischemic stroke and the role of blood-brain barrier: the dark side or the light one? *Front. Med.*, 2019, vol. 13, no. 4, pp. 420–426. <https://doi.org/10.1007/s11684-019-0688-6>.
3. Tang Y. C., Tian H. X., Yi T., Chen A. B. The critical roles of mitophagy in cerebral ischemia. *Protein Cell*, 2016, vol. 7, no. 10, pp. 699–713.

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