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Minimal changes of TNF-α and MCP-1 expression in blood serum of rats subjected to experimental cardiac arrest

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Abstract

The elucidation of various aspects of the pathomechanism of experimental cardiac arrest may contribute valuable information for the treatment of cardiac arrests in humans. The model of clinical death in rats introduced by Korpatchev et al. was used. An analysis of expression in the blood serum of TNF-α and of MCP-1 was performed as a contribution to this problem. The studied groups included rats 24, 48 hours and 14 days after experimental cardiac arrest.

Results: *The level of serum TNF-α in all studied animal groups remained unchanged after global cerebral ischemia. MCP-1 expression was decreased only in the group of rats – two weeks after the cardiac arrest.*

Conclusion: *The obtained results indicate that proinflammatory cytokines do not play a key role in the development of delayed neurodegeneration after cardiac arrest. Thus it is not so much the immunological reaction, but a neuroexcitatory mechanism that plays a decisive role in the delayed neuronal death.*

Key words: *cardiac arrest, TNF-α, MCP-1, cytokines*

Introduction

The elucidation of various aspects of the pathomechanism of experimental cardiac arrest may contribute valuable information to the treatment of human pathology [5]. In the presented study, the model of clinical death in rats, introduced by Korpachev et al. [3] has been used. In neuropathological studies on rats subjected to cardiac arrest, Mossakowski and Zelman [7] have established early neuronal changes occurring in a generalised manner, with some regional accentuation, but without a clear cut correlation to the duration of global ischemia. During the forthcoming period after ischemia, neuronal degeneration proceeds leading to neuronal losses and subsequent damage of nerve fibres [9]. The progressive nature of degeneration generated the concept of „maturation phenomenon" in cerebral ischemia [1].

It has been assumed that immunological reactions are responsible for the maturation event in cerebral ischemia. The findings of Mossakowski and Krajewski [6] demonstrating the presence of antineuronal

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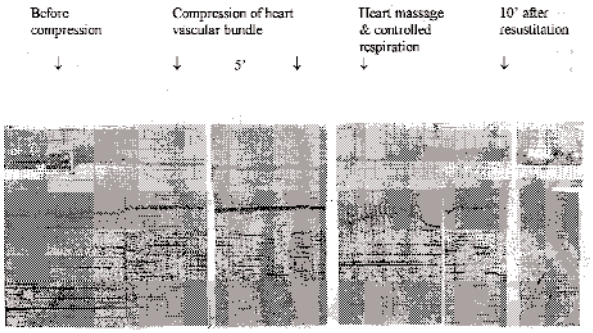

Fig. 1. Respiratory and heart function in experimental cardiac arrest. Upper part captogram line. Lower line ECG

antibodies in blood sera of rats subjected to global ischemia have led to the assumption that it is the immunological reaction that bears responsibility for the late postresuscitation encephalopathy.

Proinflammatory cytokines are believed to play a significant role in the pathomechanism of ischemic stroke. They are released in the course of ischemia by activated brain resident and peripheral blood cells and produce inflammatory processes exacerbating cerebral damage. One of the cytokines which play a crucial role in this event is the tumor necrosis factor alpha (TNF-α) [10]. The other one is the monocyte chemoattractant protein -1 (MCP-1) – a CC chemokine, both serving as mediators of monocyte recruitment and activation [4].

Aiming at contributing to the understanding of the immunological pathomechanism of postresuscitation encephalopathy we have analyzed the still unresolved problem of expression of TNF-α and MCP-1 in blood serum, which eventually could be of some relevance for the development of this syndrome.

Material and methods

Global cerebral ischemia was produced in 40 Wistar rats of either sex, 200-250 g of body weight, according to the method described originally by Korpachev et al. [3]. A midline skin incision in the chest of rats kept under light hallothane anesthesia and a special occluding hook has been inserted into the mediastinum at the level of the second intercostal segment. A complete interruption of the circulation due to compression of major cardiac vessels has thus been accomplished, leading immediately to cessation of the effective heart function. After a 5 minutes lasting compression of the vascular bundle, resuscitation was performed by external heart massage and controlled respiration (30-90 seconds) until recovery of basal vital functions was obtained (Fig. 1). The animals in groups of 10 were sacrificed after 24 and 48 hours and after 14 days – respectively.

Blood for laboratory tests was taken by heart puncture and the expression of TNF-α and MCP-1 in the serum was quantitatively evaluated by means of the ELISA method using Quantikine rat R&D Systems, Minneapolis, MN, USA.

The significance of differences was determined using the non-parametric Mann-Whitney U-test.

Results

In comparison with control animals, the serum level of TNF-α in rats subjected to global cerebral ischemia remained essentially unchanged in all the experimental study groups (24, 48 hours and 14 days after cardiac arrest). Neither were there any differences seen between the individual experimental groups. The expression of MCP-1 in rat serum after 24 and 48 hours following the provoked ischemia did not differ from that established in the serum of control animals. However, decreased levels of serum MCP-1 were found two weeks following the experimental cardiac arrest. This difference showed statistical significance also between the other experimental groups. Detailed results are presented in Table 1.

Table I. The effect of experimental cerebral ischemia on expression of TNF-α and of MCP-1 in blood serum of rats subjected to experimental cardiac arrest

	Control	24 hours after ischemia	48 hours after ischemia	14 days after ischemia
TNF-α	25.44±8.29	21.46±0.54	23.91±6.25	27.57±12.62
MCP-1	1209.33±380.36	1457±700.62	1265.16±506.58	*902.40±269.64

Number of animals in each group – 10; Results, expressed in pg/ml, are presented as means ±SD.
** Differences significant at the probability level p ≤0.05*

Discussion

Serum TNF-α level correlates in humans with both, the ischemic stroke severity and with dimension of the brain infarct, thus suggesting an involvement of this cytokine in the mechanism of stroke induced inflammatory reaction [10]. In experimental global ischemia, the dominating pathological event is neuronal degeneration, and not an evident necrosis with secondary inflammation. This difference may, at least partially, explain the lack of changes in TNF-α expression in global ischemia, versus the strong reaction seen in cerebral infarcts.

MCP-1 expression in ischemic stroke in humans happens to be increased in the CSF, but not in blood serum, [4], while in rats after global ischemia a decreased expression of this cytokine in the late periods following the cardiac arrest has been found.

The obtained results seem to corroborate the thesis that the immunological reactions provoked by the proinflammatory cytokines with the subsequent activation of intracellular signalling cascades do not play a key role in the development of delayed neurodegeneration after cardiac arrest. It is not easy to transfer experimental data directly to human pathology. Nevertheless it may be assumed that a suppression of immunological reactions cannot be used to limit the cellular damage occurring in the brain after cardiac arrest.

The pathomechanism of cardiac arrest differs from that which follows ischemia after arterial occlusion with subsequent brain infarct. In cardiac arrest, the main event is a complete cessation of blood circulation to all organs and the subsequent release from them of toxic substances [2]. However, one should rather assume that it is not so much the immunological reaction, but a neuroexcitatory mechanism that bears the responsibility for the delayed neuronal death. The search for chemical neuroprotective methods that would induce a beneficial effect safeguarding the nervous system after global ischemia may thus be substantiated, but so far obtained results of studies are still incomplete or even negative [8].

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