CYTOKINES AS MARKERS IN THE PROGNOSIS OF NEUROINFECTIONS TO THE CENTRAL NERVOUS SYSTEM (CNS)

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The viral neuroinflammation of the CNS triggers a very complex signalling cascade. In the innate immune response, the brain protects itself by releasing cytokines before the activation of the specific/adaptative immune response. The intensity of the inflammatory reaction and the effectiveness of protective mechanisms are two factors which control the course of neuroinfections. The cerebral tissue has naturally tendency to repair the lesion produced if this is achived by its components mediated by anti-inflammatory cytokines, neurotrophic factors and gliosis, and the neuronal loss is prevented. In this regard, we want to prove that neurotrophin-3 (NT-3) may exert an immunomodulating action on the IL-10 responses through mechanisms essential for the restoration of cerebral homeostasis in patients with neuroinfections in CNS. The studies of these mechanisms will make possible the initiation of treatment based on the modulation of the immunological processes involved, which will induce a significant favorable change of this diseases.

Key words: Neuroinflammation; Neurotrophins; Viral meningitis; Viral encephalitis.

INTRODUCTION

The mediators of the inflammation identified in the peripheral tissues are expressed under certain conditions into the cerebral parenchyma too.

Cytokines are regulatory proteins with multiple actions, which modulate the cellular response and in extremis lead to cell death to the all tissues of organism[1,2]. The source of the cytokines in the CNS is mainly represented by microglial cells, astrocytes but also by pericytes, intratissular infiltrating immune cells, endothelial cells and neurons[3].Therefore it is considered that the inflammatory reaction in viral neuroinfections is initiated by the activation of signaling pathway induced through the Toll like receptors (TLR) involving the activation of the NFkB and AP-1 transcription factors[4]. Experiences performed on activated human foetal microglial cell cultures have demonstrated that IL-10 inhibits the TNF-α release, minimizing the neuronal destructions which the overproduction of these cytokines would bring about[5]. The neuroimmune response in the brain includes also production of the neurotrophic factors[6]. Neurotrophins in CNS have been recognized as mediators of the neurodegenerative mechanisms as well as of the neuroprotective mechanisms in a number of CNS pathologies. Experiments on animal models demonstrated that NT-3 may be produced by glial cells as a protection response against the viral aggresion[7].

The viral meningitis and encephalitis represent also a significant cause of mobidity and mortality[8]. The herpetic encephalitis is the most frequent infection of the CNS. It may occur either as a single clinical manifestation (infection causes by HSV-1), or in the case of a viral latency, especially at the adult age[9].

In the pressent work we investigated the possible correlation between expression levels of NT-3 as compared to IL-10 in serum and cerebrospinal fluid (CFS) from patients with acute viral meningitis and encephalitis.

MATERIAL AND METHODS

Patients: 50 patients, aged between 45–75 years (58.92 ± 8.76 years), diagnostes with viral meningitis (VIR_M) and viral encephalitis (VIR_E), were investigated. Samples were taken from venous and lumbar puncture. The informed consent of the enroled patients was obtained.

The detection of IgG anti HSV-1 antibodies in serum and CFS was performed using a monoclonal antibody-based capture enzyme-linked immunosorbent assay (Alpco Diagnostics). The ratio between the IgG anti HSV-1 titre in serum and CFS was determined. In HSV-1 encephalitis the titre of the IgG anti HSV-1 in CFS, deacreased from 100 to 20.

The serum ceruloplasmin (Cp) dosage was performed by using Ravin methods. In order to differentiate between the viral and bacterial meningo-encephalitis, the cases with Cp serum levels between in normal limits (26 ± 12.1 mg/100 mL) or slightly lower were considered viral neuroinfections, as compared with those affected by bacterial neuro-disease with increased values (40.21 ± 8.51 mg/100 mL).

Quantification of the TNF-α, IL-10 and NT-3 expression. The TNF-α, IL-10 and NT-3 levels were determined by using commercial ELISA Coulter and EuroClone kits acording manufacturer indications.

Statistical analysis. Correlations analysis was performed by using the Pearson's correlation coefficient r; The minimum threshold value accepted to consider a correlation as statisticaly significant was $p \leq 0.05$.

RESULTS AND DISCUSSIONS

According to the clinical course, the patients with meningitis and encephalitis were divided as show in Table 1.

When VIR_M HSV- cases were tested, antibodies anti-echo 4 were detected in the CFS of 2 patients, while IgA adenovirus specific was found in sera from 12 patients.

NT-3 and IL-10 expresion levels. The serum and CFS level of IL-10 and NT-3 were presented in Table 2 and Table 3.

Table 1

Patients of the test group

Group	Subgroup	DEXA therapy	No. of cases	No. of days from enrolment	Clinical course
VIR_M n= 36	(HSV–)	+	22	3 days; n=8 6 days; n= 8 7 days n = 6	*Death after 10.0 ± 5.8 days* (n=10); Favourable (n=12)
		–	14	3 days; n=6 7 days; n =8	Favourable n=14
VIR_E n = 14	(HSV+)	–	14	4 days; n= 4 7 days; n= 10	*Death after10.0 ± 5.8 days;* (n=4); Death after *6.4 ± 3.4 days;* (n =10)

HSV = herpes simplex virus specific IgG: (–) absent, (+) present;

Table 2

IL-10 levels in serum and CFS detected groups under study

IL-10 serum level (pg/mL)		**IL-10 CFS level (pg/mL)**	
Control group	0.04 ± 0	***Control group***	0.01 ± 0
VIR_M HSV-DEXA+ favourable course	4.91±2.1	**VIR_M** HSV-DEXA+ favourable course	1 167.3 ± 81.6
VIR_M HSV-DEXA– medium course	46.13 ± 28.02	**VIR_M** HSV-DEXA- medium course	23.6 ± 7.10
VIR_ E HSV+ DEXA– severe course	48.51 ± 9.7	**VIR_ E HSV+DEXA- severe course**	800 ± 383

Table 3

NT-3 levels in serum and CFS detected in groups under study

NT-3 serum level (pg/mL)		**NT-3 CFS level (pg/mL)**	
Control group	5.52 ±2.54	***Control group***	12 ± 1.09
VIR_M HSV-DEXA+ favourable course	12.4 ± 5.6	**VIR_M** HSV-DEXA+ favourable course	1 17.4 ± 5.6
VIR_M HSV-DEXA– medium course	8.8 ± 4.5	**VIR_M** HSV-DEXA- medium course	18.8 ± 7.5
VIR_ E HSV+ DEXA– severe course	8.99 ± 0.75	**VIR_ E** HSV+DEXA- severe course	9.01 ± 1.55

CFS and serum TNF-α was detected on 7 days after onset in 10 patients with VIR_M (medium course) and in 14 patients with VIR-E (medium and severe course).

Correlation between the NT-3 and IL-10 expression levels. In patients VIR_M (HSV-) (DEXA+) with a medium course, a direct correlation was recorded between the NT-3 and IL-10 levels in sera (r=0.59, p=0.05) (Fig.1A). In contrast, in Fig.1B, an inverse CFS correlation between NT-3 and IL-10 was observed in patients with VIR_M (HSV-) DEXA-, with favourable course (r = -0.69, p<0.005). A direct correlation between NT-3 and IL-10 in sera of non-survivors VIR_E patients was found (r = 0.56, p=0.05) Fig. 2. Non survivors of group VIR-E exhibited exacerbated TNF-α serum levels (161± 35 pg/mL).

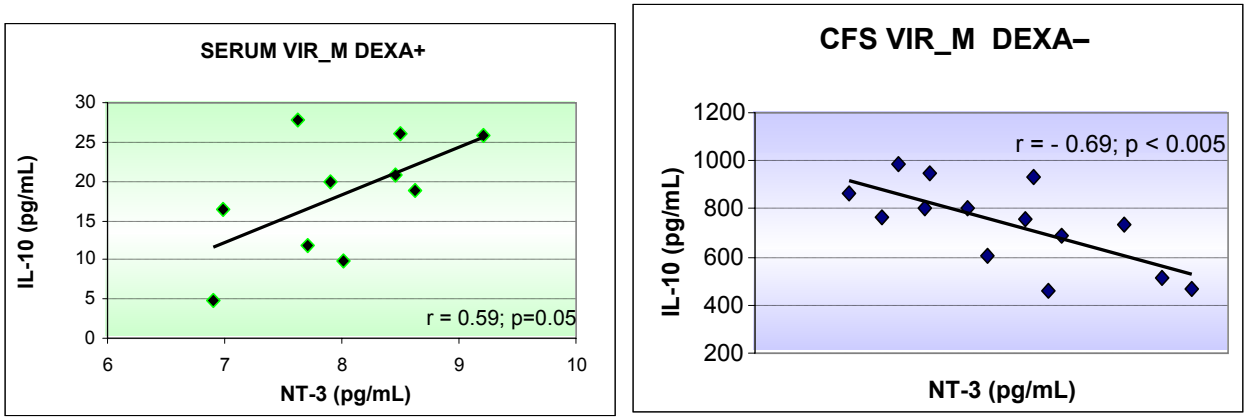

Fig.1. Correlation between serum NT-3 and IL-10 in patients with VIR_M (HSV-) DEXA+ with a medium course (A) and VIR_ M (HSV-), DEXA- with a favourable course (B).

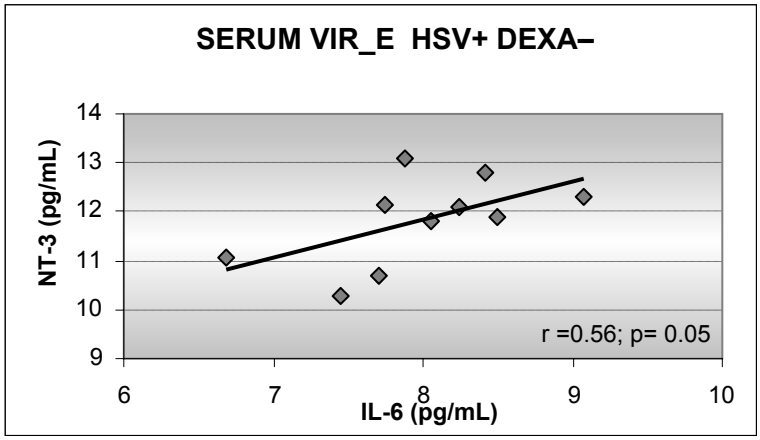

Fig. 2. Correlation between serum NT-3 and IL-10 in patients with VIR_ E (HSV+) DEXA- with a severe course.

In our study NT-3 and IL-10 cytokines were differentially secreted in terms of levels and dynamics, both in serum and CFS. Thus direct or inverse correlations have predictive values, which represent original hypothesis:

– A direct correlation between the NT-3 and IL-10 serum level and the presence of exacerbated serum level of TNF-α correspond to imminence of death in patients with a medium course, showing the effort of the organism for the limitation of the local neuro-inflammation, which finally lead to death. In the patients who died after 7 days from the onset we observed the absence of the DEXA treatment in patients with VIR_E (HSV +) in the context of TNF-α detection and an exacerbated cerulopasmin level in serum and CFS.

– An inverse correlation between NT-3 and IL-10 CFS level in patients with viral neuroinflammation with favourable course, who were not treated with DEXA, was associated with a favourable prognosis of the disease. TNF- α levels in serum and CFS were not detected after 7 days from the onset. It is suggested that, depending on the local cytokine profile, NT-3 may have a double role: pro and anti-apoptotic. The treatment with DEXA in viral neuroinfections does not modify the NT-3 secretion, but it decreases the IL-10 level, diminishes the local inflammation process, being brought about by the neuroprotective actions of NT-3. The inverse correlation between NT-3 and IL-10 suggests that NT-3 could exert on immune modulating action on the IL-10 production after the TNF-α production in patients with a favourable course after the viral neuroinflammation.

CONCLUSION

The correlations between NT-3 and IL-10 levels in serum and CFS could be used for the evaluation of disease course and efficacy of the treatment. The restore of the balance between cytokines levels could improve the disease course.

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