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CXCL1 (GRO-alpha) chemokine in acute ischaemic stroke patients

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Abstract

The inflammation accompanies and exacerbates cerebral ischaemia. The infiltrated leucocytes are thought to contribute to tissue injury in stroke patients. GRO-alpha (CXCL1) is a potent neutrophil chemoattractant which may play an important role in pathophysiology of stroke.

23 ischaemic stroke patients and 15 controls have been studied. CSF and blood sampling together with cranial CT were performed within first 24 hours of stroke. CXCL1 levels were determined by ELISA, and volume of stroke-related brain CT hypodense areas was calculated.

Stroke patients displayed significantly higher CSF CXCL1 level than controls (65.6±22.3 vs 43.8±2.3 pg/ml; p<0.01). Serum CXCL1 levels in stroke patients did not differ from controls. CSF CXCL1 level correlated positively with volume of brain CT hypodense areas (p<0.001).

The results suggest that CXCL1 may be involved in inflammatory reaction during an early phase of ischaemic stroke.

Key words: *neuroinflammation, brain ischaemia, chemokines*

Introduction

Inflammatory reaction is an important feature of the early pathophysiological response to ischaemic stroke. The reaction is initiated locally by stroke-activated brain resident cells, involves endothelial-leucocyte interactions, and includes migration of leucocytes into the site of cerebral ischaemia [5]. The brain-invading leucocytes contribute to the exacerbation of tissue injury in stroke [3,5]. Among factors involved in the activation and/or migration of leucocytes, in addition to proinflammatory cytokines and adhesion molecules, the role of chemokines, the cytokines performing chemotactic activity on leucocytes, is important [12].

Chemokines have been divided into two major subfamilies on the basis of the arrangement of the two N-terminal cysteine residues, CXC and CC, depending on whether the first two cysteine residues have an amino acid between them (CXC) or are adjacent (CC) [30]. The structural classification of the chemokines determines differences in their cell target selectivity, with CXC subfamily attracting mainly neutrophils, whereas CC subfamily acting

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primarily on monocytes, macrophages, and lymphocytes [27,30].

All the leucocyte subpopulations gradually infiltrate the ischaemic brain, and in the setting of stroke both CXC and CC chemokines have been found to be expressed by brain resident cells and/or leucocytes [12,13,27]. Experimental models of cerebral ischaemia have shown that expression of CXC and CC chemokines appears to be controlled in a highly regulated manner and have been found to precede relevant leucocyte infiltration [12,27]. Increased levels of CXC and CC chemokines have been found in body fluids of ischaemic stroke patients [13,14,17,24].

CXCL1, also known as growth-related oncogene, (GRO) alpha, is a potent neutrophil chemoattractant and activator belonging to CXC chemokines family acting mainly through CXCR2 receptors [18,30].

The role of CXCL1 in bacterial peritonitis, nephrotoxic nephritis, HIV infection and other inflammatory conditions has been shown [10,15,29]. During urosepsis, CXCL1 together with other CXC chemokines such as CXCL8 (interleukin-8 (IL-8)) and CXCL5 (epithelial neutrophil-activating protein 78 (ENA-78)) was produced within urinary tract, where they were involved in the recruitment of neutrophils [19]. Released by keratinocytes, CXCL1 plays a role in characteristic neutrophil accumulation under the stratum corneum in the highly inflamed areas of psoriatic lesions [25].

In stroke, there is early local intracerebral influx of neutrophils [5,12] and positive correlation between neutrophil accumulation and severity of the ischaemic brain damage [1]. It is therefore intriguing if CXCL1 with its neutrophil chemoattractant properties may be involved in acute phase of ischaemic stroke.

We focused the present study on two aims. The first aim was to determine CXCL1 levels in CSF and serum of ischaemic stroke patients within 24 h after the onset of the disease and to compare the results with those of a control group. The second aim was to study whether the levels of CXCL1 in ischaemic stroke patients within 24 h after the onset of the disease may be related to the observed at the same period the volume of early brain CT hypodense areas representing early stroke-related brain CT changes.

So far, CXCL1 has been studied neither in experimental nor in clinical settings of cerebral ischaemia.

Material and methods

Patients

The study involved twenty-three patients within 24 h after the onset of symptoms of first-ever ischaemic stroke aged 72.2 (10.8 years of both sexes who were diagnosed basing on history, neurological examinations, and CT of the brain. The patients were admitted between 6 and 20 h /median=12 h/ after the onset of symptoms.

All the patients had complete ischaemic stroke defined as clinical symptoms persisting for >24 h [17] and confined to middle and /or anterior cerebral artery territory. Regarding stroke risk factors, 12 patients had hypertension, 5 were smokers, 4 had diabetes mellitus, and 2 had atrial fibrilllation. To avoid enrollment of the patients with concurrent diseases or conditions interfering with inflammatory mediators expression, the following exclusion criteria were applied: presence of infections, other inflammatory, autoimmune, haematological or malignant diseases, severe renal or hepatic failure, hyperthermia, tissue injury related conditions (e.g. myocardial infarction or surgical interventions) within the last year, immunosuppression and treatment with anti-inflammatory drugs within the last six months, deep vein thrombosis, mental disorders, malnutrition, intoxications, and addiction.

The control group consisted of 15 patients diagnosed with tension headache, who were age- and sex-matched with the stroke patients. The controls did not suffer from hypertension, diabetes mellitus, and atrial fibrillation nor were they smokers. The same exclusion criteria as to the stroke patients were applied to the controls. In all control subjects CT of the brain was also performed which revealed no pathological changes.

The study was conducted according to the principles established in the Declaration of Helsinki and its amendment in Tokyo and Venice, and was approved by the Local Ethics Committee. Both stroke and control patients gave their informed consent, including consent for the lumbar puncture procedure, prior to their inclusion into the study.

Laboratory procedure

CSF and blood samples were obtained from the stroke patients within 24 h after the onset of the disease. CSF samples were centrifugated immediately

after lumbar puncture and stored at -80°C. Blood samples were allowed to clot at room temperature for 30 minutes and after having been centrifuged for 10 minutes, the obtained serum was stored at -80°C.

CXCL1 levels in CSF and serum samples were quantified by ELISA (Quantikine R&D Systems, Minneapolis, USA) according to the manufacturer's instructions and measured in duplicates. The sensitivity of the method was 10 pg/ml.

In the stroke patients the albumin CSF/serum ratios were calculated to reveal blood-brain barrier damage.

Evaluation of the volume of early brain CT hypodense areas

CT brain scans were performed parallel to orbitomeatal line using 10 mm (supratentorial) and 5 mm (infratentorial) slice thickness. The volume of early brain CT hypodense areas was calculated according to the formula based on length x depth x height areas measurement [18]. CT scans were reviewed by the neuroradiologist blinded to the clinical and laboratory data.

The measurements of the hypodense areas and calculations of their volume were performed twice with the difference not exceeding 5%.

Statistical analysis

As the obtained data on CSF and serum CXCL1 levels in the stroke patients were not normally distributed, the analysis was performed with nonparametric tests. Mann-Whitney's U test was used to compare CXCL1 levels in CSF and serum in the stroke patients with control values. Spearman's rank-order correlation test was applied to calculate the correlation between CSF CXCL1 levels and the volumes of early brain CT hypodense areas. The results are presented as mean (SD. $P<0.05$ was considered statistically significant.

Results

CSF and serum CXCL1 levels in patients within 24 h of ischaemic stroke

CSF CXCL1 level in the stroke patients was 65.6±22.3 pg/ml and was significantly higher ($p<0.01$) than that of the control group, in which the level was 43.8±2.3 pg/ml.

Serum CXCL1 level in the stroke patients was 86.9±25.1 pg/ml and did not differ significantly from that in the control group, in which the level was 85.5±31.8 pg/ml.

CSF CXCL1 levels in patients within 24 h of ischaemic stroke in relation to albumin CSF/serum ratios

CSF/serum albumin ratios were investigated in CSF and serum obtained from CSF and blood samples of ischaemic stroke patients within 24 hours after the onset of the disease. Eighteen of the 23 patients exhibited no CSF/serum albumin ratio increase (0.0047±0.0018), favouring an intact blood-brain barrier whereas the 5 remaining patients showed a small increase of CSF/serum albumin ratio (0.0105±0.0022), favouring modest blood-brain barrier damage. CSF CXCL1 levels in the group of patients without any signs of blood-brain barrier damage were 68.4±28.8 pg/ml, whereas corresponding values for patients with blood-brain barrier damage were 60.2±44.3 pg/ml (NS).

The volume of early brain CT hypodense areas in patients within 24 h of ischaemic stroke

Each ischaemic stroke patient – except one with radiologically invisible changes – presented clinically relevant single early CT hypodense area localised in cerebral hemisphere. There were no other brain CT changes.

CT analysis revealed the average hypodense areas volume to be 10.0±10.7 cm^3. The largest volume of hypodense area was 37.5 cm^3, whereas the smallest was 0.6 cm^3.

Correlation between CSF CXCL1 levels and the volume of early brain CT hypodense areas in patients within 24 h of ischaemic stroke

CSF CXCL1 levels correlated positively with the volume of early brain CT hypodense areas ($r=0.96$; $p<0.001$). The correlation is shown in Fig. 1.

Discussion

An increase in CXCL1 levels in CSF of acute ischaemic stroke patients suggests the chemokine upregulation during an early phase of stroke and is in line with the reported elevations in CSF levels of IL-8 [14,24] and monocyte chemoattractant protein-1 (MCP-1) [17] in patients with cerebral ischaemia.

As cellular origins of CXCL1 synthesis in stroke are unknown, one of the major questions is whether

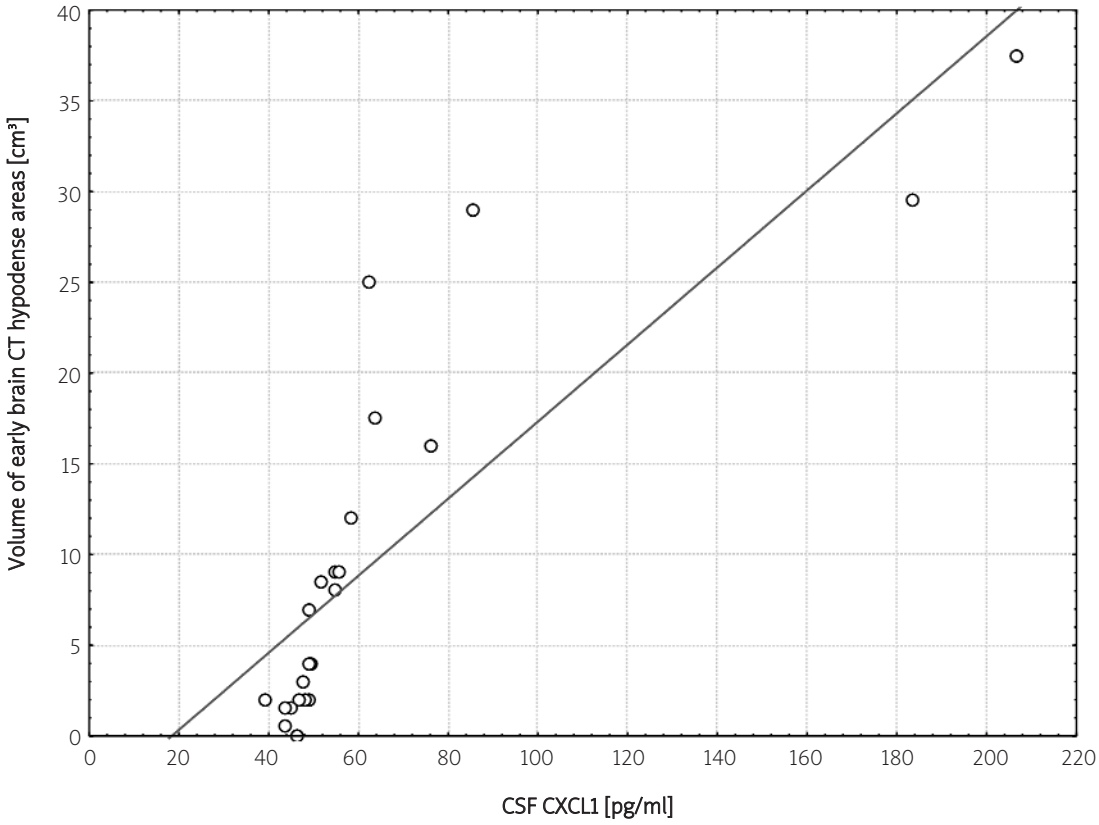

Fig. 1. Correlation® between CSF CXCL1 levels [pg/ml] in stroke patients and volume of early brain CT hypodense areas [cm³]

CXCL1 observed in CSF was produced locally by cells within the central nervous system or rather originated from the systemic compartment. The latter variant could be taken into account with the studies demonstrating a systemic increase in IL-8 mRNA expressing blood mononuclear cells as well as IL-8 levels in plasma and CSF from patients with ischaemic stroke [13,14], together with potential ability of systemically produced cytokines/chemokines to enter passively into the cerebrospinal compartment through a damaged blood-brain barrier [14,24]. The hypothesis that peripherally synthesised CXCL1 may appear in CSF of acute stroke patients requires two conditions at least: 1) high serum chemokine levels after the disease onset; and 2) higher CSF CXCL1 levels in patients with blood-brain barrier damage than CSF chemokine levels in patients without blood-brain barrier damage. We found, however, no increase in CXCL1 levels in serum of patients within 24 h after the onset of stroke, and no significant difference in CSF CXCL1 levels between stroke patients with and without blood-brain barrier damage. These findings argue against the hypothesis of early systemic production of CXCL1 with subsequent passage to CSF following stroke. Instead it advocates CXCL1 local but not systemic production during an early phase of ischaemic stroke.

A local production of CXCL1 following stroke could be a result of inflammatory response to ischaemic stroke. Indeed, accumulated data indicate that cerebral ischaemia-activated CNS cells become immunologically reactive and produce proinflammatory cytokines and chemokines which promote tissue leucocyte infiltration. The ischaemic brain-invading leucocytes may produce cytokines and chemokines which may further promote the leucocyte response [5]. It has been also shown that cultured astrocytes and microglia [11] as well as neutrophils [22] can respond to cytokines such as tumour necrosis factor-alpha (TNF-alpha) or interleukin-1 (IL-1), the molecules locally initiating

stroke-induced inflammatory reaction. Thus, both brain resident cells and brain-invaded neutrophils could be potential sources of CXCL1 local production in stroke. This suggestion is favoured by the studies reporting that mRNA [16] and protein [28] of cytokine-induced neutrophil chemoattractant (CINC), which is homologous to human CXCL1, were increased in the brain lesion areas of rats subjected to ischaemic stroke.

What role in the pathophysiology of stroke may locally produced CXCL1 perform? CXCL1, with its potent neutrophil chemotactic and activating properties, could participate – like other cytokines/chemokines released after cerebral ischaemia – in poststroke inflammation involving leucocyte accumulation within ischaemic brain. An association between local CXCL1 expression and tissue leucocyte infiltration was demonstrated following experimentally induced arthritis in rabbits, where the intra-articular CXCL1 level peaked before and at the time of maximal level of neutrophil influx into the joints [9]. Thus, early local production of CXCL1 in stroke may have an effect on the timetable of neutrophils recruitment into ischaemic brain, as they begin to migrate within hours of cerebral infarction with maximal response 24-48 h after stroke [2]. The similar role in stroke seems to be played by IL-8, another CXC chemokine with potent neutrophil-attracting activity [13,14,24]. Furthermore, there is an interference between cytokines and chemokines expressed in response to cerebral ischaemia [6,12]. Such proinflammatory cytokines/chemokines involved in brain ischaemia like TNF-alpha, IL-1, or IL-8 [6], and the chemokine CXCL1, have been shown to be reciprocally induced in vitro using cultured synovial cells or in a model of gouty arthritis in rabbits [8,9]. Moreover, CXCL1 may promote leucocyte-endothelial interactions within ischaemic brain region by the ability to induce leucocyte function associated molecule-1 (LFA-1, CD11a/CD18) dependent adhesion of neutrophils to intercellular adhesion molecule-1 (ICAM-1) [20]. Adhesion molecules, including ICAM-1, have been observed to express on ischaemic brain microvasculature in animal and human postmortem stroke studies. Its soluble forms were increased in sera of patients with acute ischaemic stroke [7,21]. Thus, ability of CXCL1 to interact with cytokines and adhesion molecules expressed after stroke may suggest that CXCL1 may potentially act in concert with these factors promoting leukocyte migration into the ischaemic brain. The relevance of CXCL1 for neutrophil accumulation is depicted by a significant reduction in neutrophil infiltration into the inflamed tissue after administration of anti-CXCL1 monoclonal antibody [9]. However, the effect of inhibiting the molecule on experimental or clinical stroke has not been yet studied.

Early brain CT hypodense areas identified within hours of stroke are thought to represent evolving ischaemic brain damage together with perilesional oedema [26]. Both these consequences of stroke are augmented by poststroke pathophysiological mechanisms, including an inflammation with tissue infiltration by leucocytes, particularly neutrophils producing a number of bioactive substances like toxic oxygen metabolites, destructive enzymes, and proinflammatory cytokines with neurotoxic properties [5,13].

A positive correlation between CSF CXCL1 levels and the volume of early brain CT hypodense areas in patients within 24 h after the onset of stroke may suggest the chemokine participation in the mechanisms contributing to an extent of tissue injury after cerebral ischaemia.

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