VIA MEDICA
ORIGINAL ARTICLE
Vol. 65, No. 1, pp. 1–5

ISSN 0015–5659
www.fm.viamedica.pl

The level of chemokine CXCL5 in the cerebrospinal fluid is increased during the first 24 hours of ischaemic stroke and correlates with the size of early brain damage

J. Zaremba[1], P. Skrobański[2], J. Losy[1, 3]

[1]Department of Clinical Neuroimmunology, Chair of Neurology, University School of Medicine, Poznań, Poland
[2]Department of Neuroradiology, University School of Medicine, Poznań, Poland
[3]Neuroimmunological Unit, Institute of Experimental and Clinical Medicine, Polish Academy of Sciences, Poznań, Poland

[Received 14 November 2005; Accepted 14 January 2006]

Inflammation is an important feature of the pathophysiological response to ischaemic stroke. The ischaemic brain-invading leukocytes, neutrophils in particular, contribute to the exacerbation of tissue injury in stroke. Chemokines are a growing family of proteins performing chemotactic activity on selective leukocyte subpopulations. Chemokines are broadly 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). CXC chemokines possessing, close to the N terminus, the amino acid sequence glutamic acid-leucine-arginine (ELR motif) specifically act on neutrophils. CXCL5 is one of the ELR-expressing CXC chemokines and is a potent neutrophil attractant and activator. The objective of the study was to detect CXCL5 levels in the cerebrospinal fluid (CSF) and sera of stroke patients and to investigate the relation between these levels and the volume of brain computed tomography (CT) hypodense areas representing early ischaemic lesions. A total of 23 ischaemic stroke patients were studied. CSF and blood sampling and brain CT were performed within the first 24 hours of stroke. The control group consisted of 15 patients with tension headache. CXCL5 levels were determined by the ELISA method. CSF CXCL5 levels in stroke patients were significantly higher in comparison with the control group (38.2 ± 18.4 pg/ml vs. 18.7 ± ± 8.2 pg/ml; $p < 0.001$). No significant differences in serum CXCL5 levels were found between the stroke patients and the control group. CSF CXCL5 levels correlated positively with the volume of early brain CT hypodense areas ($p < 0.0001$). The results suggest that CXCL5 may play a role in the inflammatory reaction during the early phase of ischaemic stroke.

Key words: chemokines, CXCL5, ENA-78, CSF, serum, stroke

INTRODUCTION

Inflammatory reaction is an important feature of the pathophysiological response to ischaemic stroke. The local intracerebral influx of leukocytes, neutrophils in particular, contributes to the exacerbation of tissue injury in stroke [4, 9].

Address for correspondence: J. Zaremba, MD, PhD, Department of Clinical Neuroimmunology, University School of Medicine, ul. Przybyszewskiego 49, 60–355 Poznań, Poland, tel: +48 061 869 14 45, fax: +48 061 869 15 83, e-mail: jlosy@amp.edu.pl

Chemokines (an abbreviation for "chemoattractant cytokines") constitute a growing family of structurally and functionally related small (8–14 kDa) proteins which perform chemotactic activity on selective leukocyte subpopulations [6, 8, 25] and which, it is now postulated, play a substantial role in the accumulation of leukocytes within an ischaemic brain lesion [15, 16].

Chemokines are broadly 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) [25]. CXC subfamily is further subdivided into those chemokines that contain the amino acid sequence glutamic acid-leucine-arginine (the ELR motif) close to the N terminus and those which do not [8]. This subdivision has functional significance, as ELR-expressing CXC chemokines chemoattract neutrophils, whereas non-ELR CXC chemokines as well as CC chemokines are chemotactic towards mononuclear cells [18].

In the settings of experimental and clinical stroke CXC and CC chemokines have been found to be expressed by brain-resident cells such as microglia, astrocytes, neurons and endothelia, and/or by the ischaemic brain-invading as well as circulating leukocytes [6, 10, 11, 16, 22]. Experimental models of cerebral ischaemia have shown that expression of CXC and CC chemokines precedes relevant leukocyte infiltration [10, 22, 23].

Increased levels of CXC and CC chemokines have been found in the cerebrospinal fluid (CSF) or peripheral blood of ischaemic stroke patients [11–13, 20].

CXCL5, originally described as an epithelial cell-derived neutrophil-activating protein consisting of 78 amino acids (ENA-78), is a potent neutrophil attractant and activator belonging to CXC chemokine subfamily which possesses the ELR motif [8]. CXCL5 exhibits extensive structural and functional similarity to other ELR-expressing CXC chemokines [8]. In particular, CXCL5 is highly homologous to CXCL6 (primarily termed granulocyte chemotactic protein (GCP-2), and these two chemokines are 80% and 88% identical at the amino acid and nucleic acid levels respectively [24, 25]. The gene for CXCL5 has been mapped to chromosome 4q12-q13 [25]. CXCL5 exerts its biological effects mainly through interaction with the CXCR2 receptor [6]. Increased CXCL5 expression has been found to be associated with neutrophil influx in several inflammatory conditions [3].

Following stroke, neutrophils are the first leukocyte subpopulation to migrate into the infarct zone. The neutrophil response progressively increases within the first 24 hours of the disease, and there is a positive correlation between neutrophil accumulation and the magnitude of the ischaemic brain damage [1]. It is therefore intriguing whether CXCL5 with its neutrophil chemoattractant properties may be involved in the early phase of ischaemic stroke.

The study was focused on two goals. The first was to determine CXCL5 levels in CSF and serum of ischaemic stroke patients within the first 24 hours of the disease and to compare the results with those of a control group. The second was to study whether the levels of CXCL5 in the stroke patients within the first 24 hours of the disease may be related to the volume of early brain (computed tomography) CT hypodense areas observed at the same period, indicating early ischaemic stroke-related brain lesions.

So far CXCL5 has been studied neither in experimental nor in clinical ischaemic stroke.

MATERIAL AND METHODS

Patients

The study involved 23 first-ever ischaemic stroke patients (mean age ± SD: 72.2 ± 10.8 years, 17 women) admitted between the 6th and the 20th hour (median = 12th h) after the onset of symptoms. CSF and serum samples were collected from each stroke patient within 30 min of admission, and the diagnosis was confirmed by brain CT performed within the next 30 min. Thus the laboratory and neuroimaging data were obtained in a uniform manner in all the stroke patients within the first 24 hours of stroke.

All the patients had complete ischaemic stroke defined as clinical symptoms persisting for > 24 hours [5]. The exclusion criteria consisted of CNS, inflammatory, immunological and malignant diseases, infections, severe renal or hepatic failure, tissue injury-related conditions within the previous year and immunosuppression, as well as treatment with anti-inflammatory drugs within the previous 6 months.

A total of 15 tension headache subjects (mean age ± SD: 70.1 ± 8.6 years, 11 women) were included as a control group. The same exclusion criteria were applied to the controls as to the stroke patients. In all control subjects CT of the brain was also performed and this revealed no pathological changes.

The study was performed on the basis of the informed consent of all the stroke patients or their relatives and of all the control subjects and the approval of the Ethics Committee of the University School of Medicine in Poznań.

Laboratory procedure

CSF samples from the stroke patients and controls were centrifuged immediately after lumbar puncture and stored at –80°C. Venous blood samples from the stroke patients and controls were allowed to clot at room temperature for 30 min, and, after being centrifuged for 10 min, the obtained serum was stored at –80°C.

CXCL5 levels in CSF and serum samples were quantified by ELISA (Quantikine R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions and examined in duplicate. The sensitivity of the method was 15.0 pg/ml.

Evaluation of the volume of early brain CT hypodense areas

Brain CT scans were carried out parallel to the orbitomeatal line using a 10 mm (supratentorial) and 5 mm (infratentorial) slice thickness. Each ischaemic stroke patient, with the exception of one with radiologically invisible changes, presented an anatomically relevant single early CT hypodense area localised in the cerebral hemisphere within the blood supply territory of the middle or anterior cerebral artery and displayed no other CT changes.

The volume (given in ccm) of early brain CT hypodense areas was calculated according to the formula based on length × depth × height (in mm) of the area measurements [19]. 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 CXCL5 levels in the stroke patients were not normally distributed, analysis was performed with non-parametric tests.

The Mann-Whitney U test was used to compare CXCL5 levels in CSF and serum in the stroke patients with control values. The Spearman rank-order correlation test was applied to calculate the correlation between CSF CXCL5 levels in the stroke patients and the volumes of early brain CT hypodense areas. The results are presented as mean ± SD and $p < 0.05$ was considered statistically significant.

RESULTS

CSF and serum CXCL5 levels in patients within the first 24 hours of ischaemic stroke and in the control group

CSF CXCL5 levels in the stroke patients were significantly ($p > 0.001$) higher in comparison with those of the control group, whereas serum CXCL5 levels in the stroke patients did not differ significantly from those in the controls (Table 1).

Table 1. CSF and serum CXCL5 levels [pg/ml] in patients within the first 24 hours of ischaemic stroke and in the control group; *$p < 0.001$

	Stroke patients	Control group
CSF	38.2 ± 18.4*	18.7 ± 8.2
Serum	1238.8 ± 309.3	1049.3 ± 430.3

The volume of early brain CT hypodense areas in patients within the first 24 hours of ischaemic stroke

Brain CT analysis revealed the average early brain CT hypodense area volume to be 10.0 ± 10.7 ccm. The largest volume of a hypodense area was 37.5 ccm, whereas the smallest was 0.6 ccm.

Correlation between CSF CXCL5 levels and the volume of early brain CT hypodense areas in patients within the first 24 hours of ischaemic stroke

CSF CXCL5 levels in patients within the first 24 hours of ischaemic stroke correlated positively with the volume of early brain CT hypodense areas observed at the same period ($r = 0.96$; $p < 0.0001$). The correlation is shown in Figure 1.

DISCUSSION

An increase in CXCL5 levels in CSF of patients within the first 24 hours of ischaemic stroke suggests

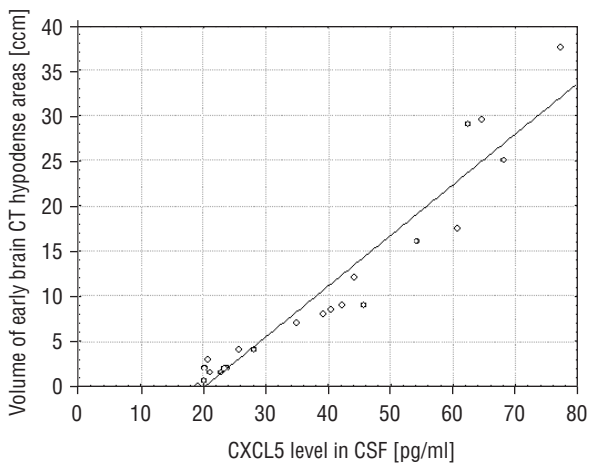

Figure 1. Correlation (r) between CSF CXCL5 levels [pg/ml] and the volume of early brain CT hypodense areas [ccm] in patients within the first 24 hours of ischaemic stroke.

chemokine upregulation during the early phase of the disease and is in line with the reported elevations in CSF levels of other CXC chemokines expressing the ELR motif, such as CXCL1 [growth-related oncogene-alpha (GRO-alpha)] [13] and CXCL8 [interleukin-8 (IL-8)] [11, 20] in patients with acute cerebral ischaemia.

An increase in CXCL5 levels in the studied body fluids of stroke patients was restricted to CSF and did not occur in the serum. Such result indicates the presence of CXCL5 over-production by cells within the central nervous system (CNS) and the absence of CXCL5 over-production by peripheral blood cells during the early phase of ischaemic stroke.

An intracerebral synthesis of CXCL5 following acute cerebral ischaemia could be a result of the inflammatory response to stroke. The response is mediated by stroke-activated CNS-resident cells and ischaemic brain-infiltrated leukocytes producing numerous inflammatory molecules including chemokines [10, 16, 22].

The cellular origins of CXCL5 within ischaemic brain are unknown. However, Albright and Gonzalez-Scarano [2] have recently shown that activated microglial cells and monocyte-derived macrophages are able to upregulate CXCL5 gene expression. Furthermore, Lu et al. [14] have reported that the transcription and translation of genes for several CXC chemokines expressing ELR motif take place in astrocytes.

CXCL5, with its intrathecal origination in stroke and potent neutrophil chemoattractant properties, appears to be, like other intracerebrally synthesised chemokines, both the product and the mediator of the neuroinflammation involving leukocyte accumulation within ischaemic brain. Hence, in view of the previous data documenting neutrophils as beginning their migration into the ischaemic tissue within hours of cerebral infarct and peaking at the 24th hour after disease onset [1, 10], the finding that there is an increase in CSF CXCL5 levels in patients within the first 24 hours of stroke could be expected to occur and suggests an involvement of this chemokine in attracting neutrophils to the sites of cerebral ischaemia.

In response to stroke, numerous chemokines and pro-inflammatory cytokines are expressed, and there is mutual interference, contributing to the development of post-stroke inflammation [9, 10, 16]. CXCL5 may act together with other chemokines, including CXCL1 and CXCL8; both of these chemokines are thought to be involved in the ischaemic brain infiltration with neutrophils [11, 13, 20]. Moreover, studies *in vitro* have demonstrated the reciprocal cross-talk between the chemokine CXCL5 and tumour necrosis factor-alpha (TNF-alpha), the cytokine-initiating neuroinflammation in stroke [7, 17].

Early brain CT hypodense areas identified within hours of stroke represent evolving ischaemic brain damage together with perilesional oedema [21]. Both these consequences of stroke are augmented by post-stroke pathophysiological mechanisms, including inflammation with tissue infiltration by leukocytes, particularly neutrophils producing a number of bioactive substances such as toxic oxygen metabolites, destructive enzymes, and pro-inflammatory cytokines with neurotoxic properties [4].

A positive correlation between CSF CXCL5 levels and the volume of early brain CT hypodense areas in patients within the first 24 hours of stroke suggests chemokine participation in the mechanisms contributing to the extent of ischaemic brain damage.

This is the first report devoted to CXCL5 in stroke, and further studies are required to confirm the detrimental role of this chemokine in acute cerebral ischaemia.

Nevertheless, the data presented a rapid increase in CXCL5 levels in the CSF of ischaemic stroke patients and the relationship between the chemokine level and the size of early brain damage suggests that CXCL5 may be involved in the inflammatory reaction accompanying acute ischaemic stroke.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Lucyna Kramer from the Department of Statistics and Information Sciences, University School of Medicine in Poznań for her kind assistance in the preparation of statistical data.

REFERENCES

1. Akopov SE, Simonian NA, Grigorian GS (1996) Dynamics of polymorphonuclear leukocyte accumulation in acute cerebral infarction and their correlation with brain tissue damage. Stroke, 27: 1739–1743.
2. Albright AV, Gonzalez-Scarano F (2004) Microarray analysis of activated mixed glial (microglia) and monocyte-derived macrophage gene expression. J Neuroimmunol, 157: 27–38.
3. Amoli MM, Larijani B, Thomson W, Ollier WE, Gonzalez--Gay MA (2005) Two polymorphisms in the epithelial cell-derived neutrophil-activating peptide (ENA-78) gene. Dis Markers, 21: 75–77.
4. Becker KJ (2001) Targeting the central nervous system inflammatory response in ischemic stroke. Curr Opin Neurol, 14: 349–353.

5. Bonita R (1992) Epidemiology of stroke. Lancet, 339: 342–347.
6. Cartier L, Hartley O, Dubois-Dauphin M, Krause KH (2005) Chemokine receptors in the central nervous system: role in brain inflammation and neurodegenerative diseases. Brain Res Rev, 48: 16–42.
7. Chandrasekar B, Melby PC, Sarau HM, Raveendran M, Perla RP, Marelli-Berg FM, Dulin NO, Singh IS (2003) Chemokine-cytokine cross-talk. The ELR+ CXC chemokine LIX (CXCL5) amplifies a proinflammatory cytokine response via a phosphatidylinositol 3-kinase-NF--kappa B pathway. J Biol Chem, 278: 4675–4686.
8. DeVries ME, Ran L, Kelvin DJ (1999) On the edge: the physiological and pathophysiological role of chemokines during inflammatory and immunological responses. Semin Immunol, 11: 95–104.
9. Emsley HCA., Tyrrell PJ (2002) Inflammation and infection in clinical stroke. J Cereb Blood Flow Metab, 22: 1399–1419.
10. Kim JS (1996) Cytokines and adhesion molecules in stroke and related diseases. J Neurol Sci, 137: 69–78.
11. Kostulas N, Pelidou SH, Kivisäkk P, Kostulas V, Link H (1999) Increased IL-1b, IL-8, and IL-17 mRNA expression in blood mononuclear cells observed in a prospective ischemic stroke study. Stroke, 30: 2174––2179.
12. Losy J, Zaremba J (2001) Monocyte chemoattractant protein-1 is increased in the cerebrospinal fluid of patients with ischemic stroke. Stroke, 32: 2695–2696.
13. Losy J, Zaremba J, Skrobański P (2005) CXCL1 (GRO--alpha) chemokine in acute ischaemic stroke patients. Folia Neuropathol, 43: 97–102.
14. Lu W, Maheshwari A, Misiuta I, Fox SE, Chen N, Zigova T, Christensen RD, Calhoun DA (2005) Neutrophil-specific chemokines are produced by astrocytic cells but not by neuronal cells. Brain Res Dev Brain Res, 155: 127–134.
15. Mennicken F, Maki R, de Souza EB, Quirion R (1999) Chemokines and chemokine receptors in the CNS: a possible role in neuroinflammation and patterning. Trends Pharmacol Sci, 20: 73–78.
16. Peters EE, Feuerstein GZ (2001) Chemokines and ischemic stroke. In: Feuerstein GZ (ed.). Inflammation and stroke. Birkhäuser Verlag, Basel, pp. 155––162.
17. Ruddy MJ, Shen F, Smith JB, Sharma A, Gaffen SL (2004) Interleukin-17 regulates expression of the CXC chemokine LIX/CXCL5 in osteoblasts: implications for inflammation and neutrophil recruitment. J Leukoc Biol, 76: 135–144.
18. Sellebjerg F, Sörensen TL (2003) Chemokines and matrix metalloproteinase-9 in leukocyte recruitment to the central nervous system. Brain Res Bull, 61: 347–355.
19. Silvestrini M, Pietroiusti A, Troisi E, Franceschelli L, Piccolo P, Magrini A, Bernardi G, Galante A (1998) Leukocyte count and aggregation during the evolution of cerebral ischemic injury. Cerebrovasc Dis, 8: 305–309.
20. Tarkowski E, Rosengren L, Blomstrand C, Wikkelsö C, Jensen C, Ekholm S, Tarkowski A (1997) Intrathecal release of pro- and anti-inflammatory cytokines during stroke. Clin Exp Immunol, 110: 492–499.
21. Von Kummer R, Allen K, Holle R, Bozzao L, Bastianello S, Manelfe C, Bluhmki E, Ringleb P, Meier DH, Hacke W (1997) Acute stroke: usefulness of early CT findings before thrombolytic therapy. Radiology, 205: 327–333.
22. Yamagami S, Tamura M, Hayashi M, Endo N, Tanabe H, Katsuura Y, Komoriya K (1999) Differential production of MCP-1 and cytokine-induced neutrophil chemoattractant in the ischemic brain after transient focal ischemia in rats. J Leukoc Biol, 65: 744–749.
23. Yamasaki Y, Matsuo Y, Matsuura N, Onodera H, Itoyama Y, Kogure K (1995) Transient increase of cytokine-induced neutrophil chemoattractant, a member of the interleukin-8 family, in ischemic brain areas after focal ischemia in rats. Stroke, 26: 318–323.
24. Zlotnik A, Morales J, Hedrick JA (1999) Recent advances in chemokines and chemokine receptors. Crit Rev Immunol, 19: 1–47.
25. Zlotnik A, Yoshie O (2000) Chemokines: a new classification system and their role in immunity. Immunity, 12: 121–127.