Eur Spine J (2002) 11 : 62–66
DOI 10.1007/s005860100306

ORIGINAL ARTICLE

H. Brisby
K. Olmarker
K. Larsson
M. Nutu
B. Rydevik

Proinflammatory cytokines in cerebrospinal fluid and serum in patients with disc herniation and sciatica

Received: 27 September 2000
Revised: 23 April 2001
Accepted: 14 May 2001
Published online: 10 July 2001

H. Brisby (✉) · K. Olmarker · K. Larsson
M. Nutu · B. Rydevik
Department of Orthopaedics,
Göteborg University,
Sahlgrenska University Hospital,
41345 Gothenburg, Sweden
e-mail: Helena.Brisby@orthop.gu.se,
Tel.: +46-31-3423908,
Fax: +46-31-823584

Abstract Proinflammatory cytokines have been identified in herniated intervertebral discs in humans, and such cytokines have experimentally been demonstrated to be important in the pathophysiological mechanisms of disc herniation. Cerebrospinal fluid (CSF) and serum concentrations of interleukin (IL)-1β, IL-6, IL-8, interferon (IFN)-γ and tumour necrosis factor (TNF)-α were investigated using the enzyme-linked immunosorbent assay (ELISA) technique in 39 patients with lumbar disc herniation and sciatica. Pain duration and pain intensity (visual analogue scale, VAS) were recorded at inclusion, and a clinical examination was performed evaluating neurological findings. The extent of disc herniation (protrusion or extrusion/sequestration) was evaluated perioperatively. Normal concentrations of IL-1β, IL-6, IFN-γ and TNF-α were present in CSF and serum in almost all patients with lumbar disc herniation. The concentrations of IL-8 in CSF were increased in 12 out of 39 patients, and these increased levels of IL-8 correlated to a short duration of pain and to more pronounced herniation (extrusion or sequestration). No relationship between IL-8 concentrations in CSF and pain intensity, positive neurological findings or a positive straight leg-raising (SLR) test was found. The observation of increased concentrations of IL-8 in CSF in patients with a short duration of symptoms supports the concept of the initial involvement of inflammatory mechanisms after a disc herniation. The finding that most of the patients with increased concentrations of IL-8 in CSF had an extrusion or a sequestration may suggest that the increase in IL-8 is related to mechanical nerve root compression, but may also indicate a biochemical effect exerted by the herniated disc on the surrounding tissue. Further studies on the potential role of IL-8 as a biomarker for disc herniation are warranted.

Keywords Disc herniation · Sciatica · Cytokines · Cerebrospinal fluid · Serum

Introduction

Nerve root pain caused by disc herniation has been suggested to be based on both chemical and mechanical factors [6, 12, 15, 19]. The chemical influence has been linked to the leakage of substances from inner part of the disc, the nucleus pulposus (NP), after rupture of the annulus fibrosus [15, 17]. Experimentally, the NP can induce an inflammatory-like reaction in and around the nerve root with increased vascular permeability, myelin changes, attraction of leukocytes and intravascular coagulation [3, 13, 17, 28]. Inflammatory mediators such as cytokines, phospholipase A_2 and nitric oxide have been suggested to be important in the pathophysiology of NP-induced nerve root injury [2, 12, 16, 18]. Increased levels of total protein and nerve injury markers have been detected in cerebrospinal fluid (CSF) in patients with disc herniation [1,

23, 24]. Such observations have created a platform for searching for other biomarkers for disc herniation in CSF. It would be even more useful to define useful biomarkers in serum, since samples are easier to obtain from serum than from CSF.

The aim of the present study was to assess whether proinflammatory cytokines could be detected in CSF and serum in patients with lumbar disc herniation and related to clinical findings.

Materials and methods

A total of 39 patients (26 men and 13 women; mean age, 39± 10 years; range, 25–64 years) undergoing discectomy were included in the study. The patients had experienced sciatic pain (mean duration, 196±233 days; range, 5–1080 days) and had disc herniation verified by computed tomography (CT) or magnetic resonance imaging (MRI). All patients filled in a questionnaire including pain duration and pain intensity according to a visual analogue scale (VAS). Neurological findings (sensory and motor deficits and reflex dysfunction) and the straight leg-raising test (SLR) were evaluated by clinical examination. The extent of disc herniation (protrusion or extrusion/sequestration) was evaluated perioperatively. CSF was obtained by a lumbar puncture two levels cranial to the planned surgical level. Both serum and CSF samples were collected just prior to surgery, centrifuged for 15 minutes at 4000 rpm and frozen at –80°C until analysis. Concentrations of interleukin (IL)-1β, IL-6, IL-8, interferon (IFN)-γ and tumour necrosis factor (TNF)-α in CSF and serum were determined using the enzyme-linked immunosorbent assay (ELISA) methodology in the 39 disc herniation patients (see below). The study was approved by the Local Human Research Ethics Committee, and informed consent was obtained from all the participating patients.

Cytokine concentrations in cerebrospinal fluid and serum

The concentrations of the five cytokines IL-1β, IL-6, IL-8, IFN-γ and TNF-α in CSF and serum were determined using commercial ELISA kits (EH2-IL1B, EH2-IL6, EH2-IL8, EH2-IFN-γ and EH2-TNFA Endogen, Woburn, MA, USA). In short, 50 µl of the serum and CSF samples (concentrated and diluted 1:10 in standard diluent containing 0.01% thimerosal) was added to each well in the pre-coated microtitre plates. Seven standardized samples and one blank were run with each series of patient samples. All samples and standard solutions were analysed in duplicate. Fifty microlitres of biotin-labelled antibody was added, and the plates were incubated for 2 h at room temperature and then washed. Streptravidin–horseradish peroxidase (HRP) solution was added to the wells (100 µl/well), and the plates were incubated for 30 min at room temperature. After washing, bound antibodies were visualized by adding a substrate solution, tetrametylbenzidine dihydrochloride (TMB). The reaction was stopped after 30 min with stop solution provided with the kit. The optical density was measured in an integrated EIA management system (Labsystems) within 30 min at two wavelengths (450 and 550 nm) to minimize optical imperfections. The data were analysed using the Genesis software program for microplate-based assays. All samples were quantitated using the standard curve for each analysed cytokine. The detection limits were 1 pg/ml for IL-1β and IL-6, 2 pg/ml) for IL-8 and IFN-γ and 5 pg/ml for TNF-α). According to the manufacturer, the intra- and interobserver variation is less than 10%. The normal levels of cytokines in serum and CSF referred to in the results section are the reference values used by the local clinical immunological laboratory in clinical routine analyses. These values are set at 2 standard deviations (SD) above the mean concentration in healthy individuals. To ensure similar standard curves in our analyses and the clinical routine laboratory analyses, a random selection of the samples was reanalysed in the clinical laboratory; the variations were less than 10%.

Statistical analyses

Distributions of the variables are given as means±SD and ranges. Fisher's exact test was used to compare proportions (dichotomous variables) between groups, and the Mann-Whitney test was used to compare continuous variables between groups. The Spearman rank correlation coefficient (r_s) was used for correlation analysis. All significance tests were conducted at the 5% significance level.

Results

The distribution of the cytokine concentrations in CSF and serum is shown in Table 1. The concentrations of TNF-α, IFN-γ and IL-1β were normal in both serum and CSF for all examined patients. The concentration of IL-6 was normal in CSF and in serum in all but one patient, who had a threefold increase (31 pg/ml) in CSF but a normal concentration in serum. IL-8 concentrations in CSF were normal (<60 pg/ml) in 27 out of 39 subjects, slightly increased (60–100 pg/ml) in 9 out of 39 patients and moderately increased (200–312 pg/ml) in 3 patients. In serum, the levels of IL-8 were 68 pg/ml in one sample and less than 15 pg/ml in the rest. The patient with the highest con-

Table 1 Distribution of cytokine concentrations in serum and cerebrospinal fluid (CSF) in 39 patients with disc herniation and sciatica

Cytokine	Normal values (pg/ml)		CSF concentration (pg/ml)		Serum concentration(pg/ml)	
	CSF	Serum	Mean±SD	Range	Mean±SD	Range
IL-1β	<4	<4	All samples below or at the detection limit of 1 pg/ml		One sample at 4 pg/ml; all other samples below or at the detection limit of 1 pg/ml	
IL-6	<10	<85	3± 5	0– 31	2± 3	0–20
IL-8	<60	<50	57±67	15–312	10±14	0–68
IFN-γ	<0.3	<5	All samples below or at the detection limit of 2 pg/ml		All samples below or at the detection limit of 2 pg/ml	
TNF-α	<4	<4	All samples below the detection limit of 5 pg/ml		All samples below the detection limit of 5 pg/ml	

IL, interleukin; IFN, interferon; TNF, tumour necrosis factor

Table 2 Clinical characteristics of the patients with increased and normal interleukin (IL)-8 concentrations in cerebrospinal fluid (CSF) and the correlation to IL-8

Concentrations of IL-8 in CSF	Straight leg-raising test*				Visual analogue scale (0–100)*		Neurological findings*				Duration of pain (days)**		Extent of herniation***	
	Positive		Negative		Mean ±SD	Range	Positive		Negative		Mean ±SD	Range	Pro-trusion (*n*)	Extrusion/ seques-tration (*n*)
	(*n*)	(%)	(*n*)	(%)			(*n*)	(%)	(*n*)	(%)				
Increased (>60 pg/ml) (*n*=12)	10	83	2	17	37±22	10–80	11	92	1	8	92±111	5– 390	1	11
Normal (<60 pg/ml) (*n*=27)	15[a]	68	7[a]	32	46±25	15–90	22	81	5	19	256±254	30–1080	13	14

[a]Data on the straight leg-raising test are missing for five patients
*, not significant; **, P=0.007; ***, P=0.017

centrations of IL-6 and IL-8 in CSF was the only patient with cauda equina compression syndrome; this patient also had the shortest duration of radiating pain (only 5 days). The distribution of VAS, neurological findings, SLR test, duration of pain and extent of the herniation between the groups of patients with increased and normal concentrations of IL-8 in CSF are shown in Table 2. No relationship was found between the concentration of IL-8 and pain intensity (VAS), neurological findings or the SLR test, but the concentrations of IL-8 showed a significant correlation with radiating pain duration (r_s=–0.48, P=0.003; Fig. 1), and sequestration or extrusion was more commonly found in the group with an increased concentration of IL-8 (P=0.017).

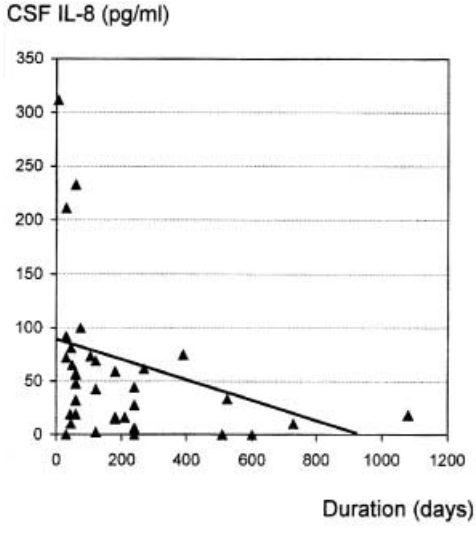

Fig. 1 The concentration (pg/ml) of interleukin (*IL*)-8 in cerebrospinal fluid (*CSF*) in relation to the duration of sciatic pain (in days) experienced by disc herniation patients at the time the samples were collected. r_s=–0.48, P=0.003

Discussion

The present study demonstrated that the concentrations of IL-1β, IL-6, IFN-γ and TNF-α in CSF and serum were normal in patients with lumbar disc herniation at the time of surgery, while IL-8 concentrations in CSF were elevated in 12 out of 39 patients; it was also shown that these elevated IL-8 concentrations showed a positive correlation with short duration of sciatic pain and a more pronounced herniation (extrusion/sequestration versus protrusion).

Cytokines are important mediators in acute (TNF-α, IL-1β, IL-6 and IL-8) and chronic (TNF-α and IL-1) inflammation [5]. Proinflammatory substances, including cytokines such as interleukins and TNF-α, are thought to be involved in the effects on the nerve roots in disc herniation [9, 10, 11, 18, 20]. Different cell types present at the site of a disc herniation, such as endothelial cells, fibroblasts and chondrocytes, have been demonstrated to express IL-6, TNF-α, IL-1α and IL-1β by immunohistochemistry [25]. Inflammatory cells such as macrophages and mast cells, cells that are known to be cytokine active, have also been shown to be present in herniated disc tissue [7, 8].

In patients with lumbar disc herniation, the permeability of the blood–nerve barrier in nerve roots may be increased, leading to extravasation of serum proteins, as demonstrated by elevated levels of protein in CSF [23, 24]. In addition, nerve tissue injury markers such as neurofilament and S-100 have been found to be increased in CSF in patients with disc herniation and sciatica [1], indicating that substances released at the site of a limited nerve root injury could be detected in the CSF. Other substances, such as cytokines, which are proposed to be involved in the pathophysiological mechanisms of disc herniation and sciatica, are therefore interesting to investigate and might be used as diagnostic and/or prognostic markers. Thus TNF-α and IL-6 have been demonstrated to be

increased in CSF and serum in patients with acute and chronic diseases of the central nervous system such as Guillan-Barré syndrome, neuropathies, multiple sclerosis and stroke and can be related to the severity of the disease [4, 14, 21, 22, 26]. IL-8 is a proinflammatory cytokine capable of attracting neutrophils as mediators of acute inflammation. Other cytokines such as IL-1β and TNF-α can stimulate cells to secrete IL-8. The increased levels of IL-8 in CSF in patients with short duration of sciatic pain in the present study indicate an early inflammatory response, which most likely decreases after a couple of months, whereafter there are no signs of elevated IL-8 (or other cytokines). The concentrations of IL-8 in CSF found in our study (mean, 57±65 pg/ml) are comparable to the concentrations in CSF and serum from healthy subjects and stroke patients in a study by Tarkowski and coworkers [27]. The mean IL-8 concentration in CSF in the control subjects was 25 pg/ml; the stroke patients showed a mean of 85 pg/ml at day 2 after onset, which decreased thereafter. The findings of a relatively acute inflammatory response in CSF after disc herniation supports previous experimental studies [3, 16], and the low concentrations measured in serum indicate that this is a local response and not a systemic inflammatory reaction. No evidence for increased concentrations of TNF-α, IL-1β or IL-6 was found in serum or CSF. This indicates that the potential involvement of these substances in disc herniation may be related to a local process that does not result in elevated levels in the CSF and serum. Alternatively, if these cytokines are involved at the onset of sciatica, the samples may have been collected too late to demonstrate any changes, as they were obtained at least 4 weeks after the onset of sciatica symptoms in all but one of the patients. Thus the absence or low levels of four of the investigated cytokines in serum and CSF do not necessarily contradict their potential role in disc herniation. However, it may be concluded that these cytokines do not seem to be of value as diagnostic markers for patients with long-standing sciatic pain symptoms.

The relationship between the extent of herniation and the IL-8 concentration might indicate that mechanical factors may cause the increase of IL-8 in CSF. On the other hand, this result might also indicate that there may be a biochemical effect induced by the NP, since substances from the inner part of the disc (NP) might reach the surrounding tissue to a greater extent if the herniation is not covered with annulus fibrosus and ligament tissue.

A limitation of the present study is that no control group was included. This was because of the difficulties of obtaining CSF from healthy controls by lumbar puncture. The normal concentration of various proinflammatory substances in CSF and serum are documented and used for clinical routine investigations of neurological and infectious conditions involving the nervous system. The finding of increased concentrations compared with reference intervals used at the local clinical laboratory is therefore highly interesting, even without comparison with a control group.

Conclusion

The present study demonstrated an increase of CSF concentrations of IL-8 in disc herniation patients with short duration of radiating nerve root pain, supporting the hypothesis that inflammation is involved in sciatic pain and disc herniation. Further investigations on patients with short duration of symptoms regarding the potential role of IL-8 as a biomarker for disc herniation are warranted.

Acknowledgements The study was supported by grants from the Swedish Medical Research Council (project no 8685), IngaBritt and Arne Lundberg Research Foundation, Greta and Einar Askers Research Foundation, Gothenburg Medical Society and Sahlgrenska University Hospital Foundation.

References

1. Brisby H, Olmarker K, Rosengren L, Cederlund C-G, Rydevik B (1999) Markers of nerve tissue injury markers in the cerebrospinal fluid in patients with lumbar disc herniation. Spine 24: 742–746
2. Brisby H, Byröd G, Olmarker K, Miller V M, Aoki Y, Rydevik B (2000) Nitric oxide as a mediator of nucleus pulposus-induced effects on spinal nerve roots. J Orthop Res 18:815–820
3. Byröd G, Otani K, Brisby H, Rydevik B, Olmarker K (2000) Methylprednisolone reduces the early vascular permeability increase in spinal nerve roots induced by epidural nucleus pulposus application. J Orthop Res 18:983–987
4. Druvolic J, Mostarica-Stojkovic M, Levic Z, Stojsavljevic N, Pravica V, Mesaros S (1997) Interleukin-12 and tumor necrosis factor-alpha levels in cerebrospinal fluid of multiple sclerosis patients. J Neurol Sci 147:145–150
5. Feghali CA, Wright T (1997) Cytokines in acute and chronic inflammation. Front Biosci 2:d12–26
6. Goupille P, Jayson MI, Valat JP, Freemont AJ (1998) The role of inflammation in disk herniation-associated radiculopathy. Semin Arthritis Rheum 28:60–71
7. Grönblad M, Virri J, Tolonen J, Seitsalo S, Kaapa E, Kankare J, Myllynen P, Karaharju EO (1994) A controlled immunohistochemical study of inflammatory cells in disc herniation. Spine 19:2744–2751
8. Habtemariam A, Virri J, Grönblad M, Seitsalo S, Karaharju E (1999) The role of mast cells in disc herniation inflammation. Spine 24:1516–1520
9. Igarashi T, Kikuchi S, Shubayev V, Myers RR (2000) Exogenous TNF-α mimics nucleus pulposus-induced neuropathology: molecular, histologic, and behavioral comparisons in rats. Spine 25:2975–2980
10. Kang JD, Georgescu HI, Larkin L, Racic M, Evans CH (1996) Herniated lumbar intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6 and prostaglandin E2. Spine 21:271–277

11. Kang JD, Stefanovic-Racic M, McIntyre LA, Georgescu HI, Evans CH (1997) Toward a biochemical understanding of human intervertebral disc degeneration and herniation. Contributions of nitric oxide, interleukins, prostaglandin E2, and matrix metalloproteinases. Spine 22:1065–1073
12. Kawakami M, Tamaki T, Hayashi N, Hashizume H, Nishi H (1998) Possible mechanism of painful radiculopathy in lumbar disc herniation. Clin Orthop 351:241–251
13. Kayama S, Olmarker K, Larsson K, Sjögren-Jansson E, Lindahl A, Rydevik B (1998) Cultured, autologous nucleus pulposus cells induce functional changes in spinal nerve roots. Spine 23: 2155–2158
14. Maimone D, Gregory S, Arnason BG, Reder AT (1991) Cytokine level in the cerebrospinal fluid and serum of patients with multiple sclerosis. J Neuroimmunol 32:67–74
15. Olmarker K, Rydevik B, Nordborg C (1993) Autologous nucleus pulposus induces neurophysiologic and histologic changes on porcine cauda equina nerve roots. Spine 18:1425–1432
16. Olmarker K, Byröd G, Cornefjord M, Nordborg C, Rydevik B (1994) Effects of methylprednisolone on nucleus pulposus-induced nerve root injury. Spine 19:1803–1808
17. Olmarker K, Rydevik B, Nordborg C (1996) Ultrastructural changes in spinal nerve roots induced by autologous nucleus pulposus. Spine 21:411–414
18. Olmarker K, Larsson K (1998) Tumor necrosis factor α and nucleus-pulposus-induced nerve root injury. Spine 23: 2538–2544
19. Olmarker K, Myers RR (1998) Pathogenesis of sciatic pain: role of herniated nucleus pulposus and deformation of spinal nerve root and dorsal root ganglion. Pain 78:99–105
20. Rand N, Reichert F, Floman Y, Rotshenker S (1997) Murine nucleus pulposus-derived cells secrete interleukins-1-β, -6, and –10 and granulolocyte-macrophage colony-stimulating factor in cell culture. Spine 22:2598–2602
21. Rentzos M, Nikolaou C, Rombos A, Voumvorakis K, Segeditsa I, Papageorgiou C (1996) Tumour necrosis factor alpha is elevated in serum and cerebrospinal fluid in multiple sclerosis and inflammatory neuropathies. J Neurol 243:165–170
22. Sharif MK, McLean B, Thompson EJ (1993) Elevated serum levels of tumor necrosis factor-alpha in Guillain-Barre syndrome. Ann Neurol 33:591–596
23. Skouen JS, Larsen JL, Vollset SE (1993) Cerebrospinal fluid proteins as indicators of nerve root compression in patients with sciatica caused by disc herniation. Spine 18:72–79
24. Skouen JS, Larsen JL, Vollset E, Gronning M (1994) Elevated cerebrospinal fluid proteins in sciatica caused by disc herniation. J Eur Spine 3:107–111
25. Takahashi H, Sugaro T, Okazima Y, Motegi M, Okada Y, Kakiuchi T (1996) Inflammatory cytokines in the herniated disc of the lumbar spine. Spine 21:218–224
26. Tarkowski E, Rosengren L, Blomstrand C, Wikkelso C, Jensen C, Ekholm S, Tarkowski A (1995) Early intrathecal production of interleukin-6 predicts the size of brain lesion in stroke. Stroke 26:1393–1398
27. Tarkowski E, Rosengren L, Blomstrand C, Wikkelso C, Jensen C, Ekholm S, Tarkowski A (1997) Intrathecal release of pro- and anti-inflammatory cytokines during stroke. Clin Exp Immunol 110:492–499
28. Yabuki S, Kikuchi S, Olmarker K, Myers RR (1998) Acute effects of nucleus pulposus on blood flow and endoneurial fluid pressure in rat dorsal root ganglia. Spine 23:2517–2523