Increased cerebrospinal fluid concentrations of soluble Fas (CD95/Apo-1) in hydrocephalus

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Abstract

Background and aims—The ventricular enlargement observed in children with chronically raised intracranial pressure (ICP) causes a secondary loss of brain tissue. In animal studies of hydrocephalus, programmed cell death (apoptosis) has been found as a major mechanism of neuronal injury. One of the regulators of the apoptotic cell death programme is the receptor mediated Fas/Fas ligand interaction.

Methods—The apoptosis regulating cytokines soluble Fas (sFas) and soluble Fas ligand (sFasL) were studied in the cerebrospinal fluid (CSF) of 31 hydrocephalic children undergoing shunt surgery for symptomatic hydrocephalus and 18 controls.

Results—High concentrations of sFas were observed in children with hydrocephalus (median 252 ng/ml); in controls sFas was below the detection limit (0.5 ng/ml). sFasL was undetectable in all but one sample.

Conclusion—High concentrations of sFas in the CSF of children with hydrocephalus suggest intrinsic sFas production, potentially antagonising pressure mediated Fas activation.

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Keywords: sFas; sFas ligand; apoptosis; hydrocephalus; ventricular dilatation

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Thus, hydrocephalus may be added to the growing list of disorders associated with inappropriate cell death in the developing brain, but the molecular mechanisms have not been completely elucidated.

Apoptosis is biochemically and genetically programmed cell death that is distinct from necrosis because it requires time, energy and, in some cases, new gene transcription and translation. A variety of extra- and intracellular signals that regulate the apoptotic cell death programme have been identified.^{8–10} The interaction between the Fas (CD95/Apo-1) cell surface receptor and its death inducing ligand (FasL) play an important role in triggering the apoptotic pathway.¹¹ Both Fas and FasL exist as membrane bound and soluble forms. Soluble Fas (sFas) is generated by differential splicing via deletion of an exon encoding the transmembrane domain of Fas. It blocks cell death by inhibiting the interaction between Fas and Fas ligand on the cell surface and thereby serves as an apoptosis regulating protein.^{12–13} In contrast, soluble Fas ligand (sFasL) is generated by proteolytic cleavage of the membrane bound form and is a less potent death inducing cytokine than FasL.^{14–15} The regulation of sFas and sFasL synthesis is subject to intensive investigations in cell culture and animal models.

Fas has been detected on a variety of cell types including neuronal cells. In the central nervous system, Fas expression has been shown on neurones, astrocytes, and oligodendrocytes in several disorders.^{16–19} Importantly, Fas upregulation has recently been shown in the adult and in the developing rodent brain following hypoxia-ischaemia.^{20–22} However, the role of the Fas/Fas ligand system in chronic hypoxic states, as present in the hydrocephalic brain remains unclear.

Our aim was to analyse the intrathecal release of sFas and sFasL as potentially regulating elements of ongoing apoptotic cell death, and to relate these to clinical findings in hydrocephalus.

Subjects and methods

PATIENT SELECTION

The study was approved by the Ethical Committee of the Ludwig Maximilians University, Munich, Germany.

Ventricular CSF was obtained from 31 children (aged 1 month to 16 years, median 7.8 years) undergoing shunt surgery between 1996 and 1998. Seven of these patients underwent surgery more than once, so repeated samples were taken (total number of CSF samples: 42).

Hydrocephalus is a pathological enlargement of the cerebral ventricles that results from an impaired flow of cerebrospinal fluid (CSF). The ventricular enlargement observed in children with chronically raised intracranial pressure (ICP) reflects both a passive response to accommodate to excessive CSF and a secondary loss of brain tissue.

In animal studies and in human hydrocephalic brains, atrophy of the periventricular white matter, and to some extent the grey matter, as well as damaged axons are consistent findings. Coincident with axonal damage, dying oligodendrocytes and cortical neurones have been found by ultrastructural examination.^{1–5} Several injurious stimuli, such as chronic ischaemia and a combination of mechanical and metabolic factors and loss of appropriate axonal contacts have been implicated in brain tissue loss in hydrocephalus.^{6–7} In animal studies by Del Bigio and Zhang, cell death in the immature hydrocephalic brain has been suggested both as necrotic and apoptotic.³

Table 1 Laboratory findings in the CSF of children with hydrocephalus and in controls

	Hydrocephalic children	n	Controls	n
sFas (ng/ml)	252 (103–808)	36	<0.5*	18
sFasL (ng/ml)	<0.1*	41	<0.1*	18
Protein (mg/dl)	207 (114–418)	35	24.5 (21–27.8)	18
Leucocyte count (cells/ μ l)	6 (1–22)	35	2 (0–5)	18

Results expressed as median (IQR).

*Concentrations were below the detection limit (<0.5 ng/ml for sFas, <0.1 ng/ml for sFasL).

ICP was measured during surgery. Patients had hydrocephalus associated with spinal dysraphism (n = 7), intracerebral haemorrhage (n = 16), Arnold–Chiari malformation (n = 3), arthrogryposis multiplex congenita (n = 1), subarachnoid cyst (n = 1), listeria meningitis (n = 1), toxoplasmosis (n = 1), and brain tumour in remission (n = 1). In all patients ventricular dilatation and increased ICP was confirmed by physical examination and neuroimaging.

Patients were evaluated by standardised examination for symptoms of ICP, motor and sensory deficit, peripheral reflexes, ocular fundus, muscular tone, cranial nerve function, and psychomotor development. Increased ICP was initially symptomatic on 36/42 occasions with seizures (n = 21), vomiting (n = 26), bulging fontanelle (n = 19), mental alteration (n = 14), headaches (n = 7), and papilloedema (n = 7). Among patients with previous shunt implantations, shunt dysfunction (16/18) and disconnection (2/18) were evident at surgery. In addition, cerebral computed tomography, magnetic resonance imaging, and/or ultrasound scans were obtained before surgery; all patients had ventricular enlargement. No patient had a history of autoimmune or progressive malignant disease.

CSF samples from 18 children without neurological deficit (aged 1 day to 16 years, median 8 years) who underwent lumbar puncture for the exclusion of meningitis served as controls. For practical reasons it was impossible to obtain samples from the same source (ventricular/lumbar CSF) in the study population and in controls. Some controls experienced vomiting (n = 6), fever (n = 4), singular febrile seizures (n = 2), and headaches (n = 1). Fundus examination was normal in all controls.

Serum C reactive protein (CRP), ventricular CSF leucocyte count, CSF protein concentration, and bacterial culture were obtained in all subjects.

SFAS AND SFAS LIGAND ASSAYS

sFas concentrations were determined with a commercial second generation sandwich enzyme linked immunosorbent assay (ELISA; Medical & Biological Laboratories, Japan) using a polyclonal coating antibody, binding to sFas amino acid residues 305–319, and a monoclonal detecting antibody, binding to residues 110–120 of spliced sFas at a detection limit of 0.5 ng/ml.^{12–23} sFasL concentrations were determined with a sandwich ELISA (Medical & Biological Laboratories, Japan) using monoclonal antibodies for coating and

Figure 1 CSF protein content and sFas concentrations in children with hydrocephalus (n = 42) and in controls (n = 18).

binding (clones 4H9 and 4A5) at a detection limit of 0.1 ng/ml.²⁴

Protein concentrations in CSF were determined by the micro bicinchoninic acid method.²⁵ All assays were performed at least in duplicate. A microplate photometer (Dynatech MR5000, Denkendorf, Germany) was used for analysis of 96 well microtitre plates.

Statistical analyses and graphics were produced with S-Plus 4.5 and entailed univariate statistics, robust statistics, linear regression analysis, and the Mann–Whitney U test for comparing distributions.

Results

CSF leucocyte counts were low in patients and controls, exceeding 100 per μ l in two cases only with hydrocephalus (table 1). Forty eight hour bacterial cultures from all CSF samples were sterile. Serum CRP concentrations were only slightly high, and did not differ in patients (median 0.9 (interquartile range, IQR 0.6–1.4)) and controls (0.9 (0.6–1.3) mg/dl).

From a total number of 31 patients, 31 initial and 11 repeated samples were taken. sFas concentrations were high in 26/31 and were not detectable in 5/31 initial samples. In 10/11 repeated samples, sFas was high. In controls, neither sFas nor sFasL were detectable (table 1). Restricting the analysis to initial samples did not yield results essentially different from those analysing all samples.

Figure 1 shows the correlation of sFas concentrations and protein concentrations ($r_s = 0.48$, $p = 0.02$). sFas did not correlate with CSF leucocyte count ($r_s = 0.3$) or with age ($r_s = 0.4$) (data not shown). In patients with CSF protein concentrations either above or below the median protein concentration (207 mg/dl), sFas concentrations were 71 (184–266) ng/ml (n = 17) and 115 (497–1019) ng/ml (n = 18) respectively (difference not significant; $p = 0.09$). In those patients with ventricular CSF leucocyte counts in the range of the control lumbar CSF leucocyte counts (0–5 per μ l) (n = 16), sFas concentrations did not differ from sFas concentrations in patients with increased leucocyte numbers (n = 19, $p = 0.23$).

sFasL was undetectable in all but one sample of a hydrocephalic patient (298 ng/ml) with corresponding sFas concentrations of 72 ng/ml.

As there were disparate subgroups of patients included in this study, sFas was analysed in relation to the underlying diagnoses for hydrocephalus as follows: (1) intracerebral haemorrhage; (2) other cause of mechanical obstruction than haemorrhage (Arnold–Chiari, subarachnoid cyst, inflammatory or neoplastic disease). Analysis in the above described subgroups and in all patients did not reveal any significant difference in sFas and sFasL concentrations.

Furthermore, the magnitude of ICP, as measured during shunt surgery and as indicated by the cited symptoms and the acuity of presentation did not correlate with leucocyte count, or protein, sFas, and sFasL concentrations. In addition, the degree of ventricular enlargement as detected by imaging studies was not related to the concentrations of cytokines or protein, or leucocyte count.

Discussion

A growing body of evidence points out the role of apoptosis as contributing to brain cell death. It has recently been shown that Fas, one of the key inducers of apoptosis, is upregulated following hypoxia–ischaemia of the developing rat brain.²² Our study shows high intrathecal concentrations of the soluble form of Fas (CD95/Apo-1) in the CSF of children with hydrocephalus, a chronic hypoxic–ischaemic event.

sFas, a cytokine with antiapoptotic properties, has been detected in several neurological diseases, particularly in autoimmune disorders, neurodegenerative, and malignant diseases.^{26–28} It has been further identified as a marker in the serum of patients with alcoholic liver disease and chronic obstructive lung disease, indicating a correlation between the severity of the disorder and ongoing apoptotic organ cell death.^{29–30}

In a recent study of acute ischaemic brain injury, Tarkowski *et al* showed decreasing concentrations of sFas in the CSF of stroke patients during the acute clinical course. In addition, the amount of sFas was negatively correlated with the degree of neurological deficit and with the infarct volume.³¹ The authors suggested an initially high intrinsic production of sFas to protect residual CNS cells and a subsequently increased receptor mediated consumption of this molecule. In contrast to an acute stroke, hydrocephalus is a chronic event with, in most cases, immediate clinical recovery after reduction of ICP. The findings in stroke patients combined with our observations suggest that in chronic disease states, upregulation of antiapoptotic cytokines might prevent severe damage.

The detection of sFas in the CSF of hydrocephalic children supports the notion of the activation of an intrinsic protection system for brain cells. Moreover, the sFas concentrations detected in the CSF of our patients were well above the previously published sFas

concentrations in CSF and serum in patients with other neurological disorders and in control samples measured with our assay system.^{27–31–32} There was a discrepancy between the majority of samples containing high concentrations of sFas and the very few samples below the detection limit. It is assumed that sFas concentrations display a logarithmic distribution, only the top of which is detected by ELISA techniques.

Protein concentrations were higher in patients than in controls. It has to be taken into account that, unavoidably, samples from controls were obtained by lumbar puncture. Gradients certainly exist in various protein and other metabolic factors dependent on the underlying disease.³³ However, the correlation of sFas concentrations to protein concentrations in both groups indicated that sFas concentrations are not merely a result of a disturbed blood–brain barrier (fig 1).

Increased sFas concentrations have also been found in the CSF of patients with acute bacterial meningitis but not in patients with viral meningoencephalitis, with invading leucocytes considered to be the main source of sFas.³² The lack of a correlation between leucocyte accumulation and sFas concentrations in the CSF points to central nervous system cells as the main source of sFas production. As neurones, oligodendrocytes, and astrocytes all have the potential to transcribe the Fas gene, the exact origin of sFas in the hydrocephalic brain remains to be determined.^{16–19}

Recent studies suggest that the soluble form of the proapoptotic Fas ligand can cause systemic tissue damage with high sFasL concentrations reported in alcoholic liver damage, malignancies, and neurological diseases.^{24–29–34–35} In contrast to Ertel *et al*, who found sFasL to be increased in the CSF of patients with acute severe head trauma and increased intracranial pressure,³⁴ we did not detect any sFasL, except in one patient. Their findings suggest the production of sFasL as an autoregulatory defence mechanism of the host against Fas bearing leucocytes, invading the brain after head injury.³⁴ In the hydrocephalic brain there is no significant inflammation, possibly explaining our observations.⁵

None of the previous studies employed the simultaneous measurement of sFas and sFasL. Kinetic studies, possibly in combination with other cytokines, might be helpful for monitoring ongoing brain damage and intrinsic protection systems after an insult.

We conclude that our findings may indicate a propensity towards apoptosis and a pivotal role for the Fas/FasL pathway in the pathophysiology of hydrocephalus. Monitoring of sFas in the CSF might provide additional information in cases where clinical symptoms of raised intracranial pressure are less clear, for example in slit ventricle syndrome. In addition, there is an unfortunate lack of appropriate data on mechanisms of cell death in hydrocephalus; as infants and children with this disorder survive for a long time, neuropathological evidence is rare. Furthermore, animal studies will be

needed to determine exactly the involvement of apoptosis and the role of the Fas/FasL system in the hydrocephalic brain.

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