SHORT REPORT

High concentrations of procollagen propeptides in chronic subdural haematoma and effusion

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Procollagen propeptides increase in the CSF after subarachnoid haemorrhage, reflecting increased collagen synthesis in the arachnoid. We studied the induction of dural collagen synthesis after cerebral trauma by measuring the carboxyterminal propeptide of type I procollagen (PICP) and the aminoterminal propeptide of type III procollagen (PIIINP) in 17 subdural haematoma or effusion fluid samples obtained at operation on days 10–85 after head trauma. The concentration of PICP was 78-fold higher and that of PIIINP 156-fold higher, relative to that in the serum. These results indicate that meningeal trauma is followed by a long lasting increase in dural collagen synthesis, and suggest that enhanced synthesis of the various extracellular matrix components may have a role in the development of chronic subdural haematoma or effusion.

A fluid containing space has been postulated at the dura-arachnoid junction, but many studies offer compelling evidence that no such subdural space exists.^{1–3} The putative space is said to be located within the dural border cell (DBC) layer, which is continuous with the meningeal dura externally and with the arachnoid internally.^{1–3} This layer is characterised by flattened fibroblasts, a paucity of cell junctions, a lack of extracellular collagen, and enlarged extracellular spaces containing non-filamentous, amorphous

material.^{1–3} These features make the DBC layer structurally weaker than the external portions of the dura and the arachnoid, and under some pathological conditions it can be cleaved open and become filled with blood or effusion fluid.³

We have previously found that the meninges are a site for active collagen synthesis.^{4–6} The cerebrospinal fluid (CSF) concentration of the carboxyterminal propeptide of type I procollagen (PICP) is 73 µg/l and that of the aminoterminal propeptide of type III procollagen (PIIINP) is 3.5 µg/l, being similar to concentrations in serum.^{4–6} These concentrations change in a time dependent manner in the CSF of patients with subarachnoid haemorrhage (SAH).^{4–7} Procollagen propeptides recovered from the CSF of patients with SAH indicate that blood induces a fibroproliferative reaction, and probably reflect collagen synthesis in the arachnoid,^{4–6} although a definite fibroproliferative response has also been detected in the dura of the rat after experimental SAH.⁵

The subdural haematoma cavity is formed within the DBC layer, and remnants of DBCs are usually found on the adjacent surfaces of the dura and the arachnoid.³ The rate of dural collagen synthesis may therefore be estimated by analysing subdural fluids. For this purpose, we obtained chronic subdural

Abbreviations: CSF, cerebrospinal fluid; DBC, dural border cell; PICP, propeptide of type I procollagen; PIIINP, propeptide of type III procollagen; SAH, subarachnoid haemorrhage

Table 1 Demographic characteristics, clinical features, and computed tomographic findings for 17 patients with chronic subdural haematoma or effusion

Patient no.	Sex/age (y)	Preoperative clinical findings	Location of SDH/effusion	Thickness of SDH/effusion in CT (mm)	Operation after trauma (days)
Chronic subdural haematoma					
1	M/76	D, IC, MI,	R	20	10
2	M/89	D, IC, HP, MI	R	20	60
3	M/82	D, IC, HP	R	30	52
4	M/78	IC, MI, SI	R, L	20, 20	14
5	M/54	HP	R	15	67
6	M/46	D, IC, HP, MI	L	25	85
Chronic subdural effusion					
7	M/72	D, IC, SI,	R, L	13, 10	19
8	M/71	D, IC, HP, SI	R, L	12, 10	30
9	F/47	HA	R	10	15
10	M/39	D, IC, HP	R, L	10, 12	31
11	F/72	D, IC, MI	R, L	12, 10	25
12	F/79	D, IC, MI	R, L	15, 18	30
13	M/67	D, MI	R	12	Unknown
Postoperative subdural effusion					
14	F/73	HP	R	15	25*
15	M/81	D, MI	R	15	20*
16	M/81	D, MI	L	20	61*
17	F/83	D, IC, HP, MI	L	30	31*

D, disorientation; HA, headaches; HP, hemiparesis; IC, impairment of consciousness; MI, memory impairment; SI, speech impairment; SDH, chronic subdural haematoma; R, right; L, left.

The aetiology of subdural haematoma or effusion was unknown in patients 13, 16, and 17, and consisted of head trauma in all the remaining patients.

*Time lapse between the first craniotomy and reoperation.

Table 2 Concentrations of PICP and PIIINP in the subdural fluid of patients with chronic subdural haematoma or subdural effusion and in serum

Sample	No. patients (n)	PICP (mg/l)	PIIINP (µg/l)	PICP/PIIINP
Subdural fluid				
Chronic subdural haematoma	6	10.9 (4.06–47.6)	567 (490–1360)	17 (8–35)
Chronic subdural effusion	7	4.90 (2.60–16.6)	578 (140–871)	14 (8–20)
Postoperative subdural effusion	4	8.33 (6.18–11.2)	411 (205–492)	22 (13–50)
Serum				
Controls	22	0.104 (0.064–0.224)	2.7 (2.0–5.0)	39 (16–102)
Patients with subdural fluid	17	0.087 (0.049–0.203)	3.2 (1.9–19.1)	32 (11–48)
Cerebrospinal fluid				
Controls	21	0.073 (0.057–0.106)	3.5 (2.4–5.5)	21 (16–29)

All values are medians (range). The data for the controls are from our previous reports.^{4,6}

haematoma and effusion fluid samples at surgery, and measured the concentrations of PICP and PIIINP in them.

PATIENTS AND METHODS

Patients

Seventeen patients (12 men, five women) who underwent surgical treatment of chronic subdural haematoma or effusion were recruited (table 1). They were required to be free of rheumatoid arthritis, other connective tissue diseases and liver disease, not to have been treated with corticosteroids, and not to have had previous head traumas. Their mean age was 70 years (range 39–89). The most common symptoms on presentation were disorientation, impairment of consciousness, impairment of memory, mild to moderate weakness, or hemiparesis and impairment of speech. The extent of the subdural haematoma or effusion was evaluated by measuring the maximal thickness of the subdural fluid collection in the diagnostic computed tomography scan.

The patients were operated on under local or general anaesthesia. The decompression procedure consisted of a conventional burr hole, and after dividing the membranes, the haematoma or effusion fluid was aspirated into polypropylene tubes. Seven samples were clear yellow in colour, representing chronic subdural effusion, and six were dark brown in colour, representing typical chronic subdural haematoma (table 1). Four samples were taken at reoperation for chronic subdural haematoma and were classified as postoperative effusion on the basis of their yellow colour. A parallel blood sample was taken from the patients, and blood samples were also obtained from 22 controls (nine men, 13 women; mean age 41 (SD 14) years) with no definable neurological or other previously known chronic disease. The protocol was approved by Ethics Committee of Seinäjoki Central Hospital.

Methods

Immediately after evacuation of the subdural haematoma or effusion, portions of the intraoperatively aspirated material and the blood samples were centrifuged at 3000 rpm for 10 minutes; the supernatant was stored at –75°C until assayed. After appropriate dilution with phosphate buffered saline, the samples were assayed for the carboxyterminal propeptide of type I procollagen (PICP) and the aminoterminal propeptide of type III procollagen (PIIINP) using specific radioimmunoassays (Orion Diagnostica, Turku, Finland). The propeptides are released in a 1:1 ratio during synthesis of the respective procollagens,⁸ and can thus be used as indicators of the rate of collagen synthesis.⁹ Non-parametric tests were used in the statistical analysis, since the sample groups were small and most of the distributions were found to be skewed.

RESULTS

Notably high concentrations of PICP and PIIINP were found in the subdural fluid (table 2), but serum concentrations did not

differ between the patients and the controls. The median concentration of PICP in the 17 samples of subdural fluid was 7.42 mg/l (range 2.60–47.6 mg/l), 78 times higher than that in the parallel serum samples. Correspondingly, the concentration of PIIINP was 0.50 mg/l (range 0.14–1.36 mg/l), 156 times higher than that in the serum. The PICP/PIIINP ratio in the subdural fluid was 15 (range 8–50), being similar to that in the CSF of controls (table 2), whereas the ratio was 32 (range 11–48) in parallel serum samples ($p = 0.02$ for difference between subdural fluid and serum; Wilcoxon signed rank test). The highest propeptide concentrations were 47.6 mg/l for PICP and 1.36 mg/l for PIIINP, in patient 2 with subdural haematoma. The subdural fluid/serum ratio of this patient was 548 for PICP and 504 for PIIINP. There were no significant differences in propeptide concentrations between the chronic subdural haematoma, post-traumatic subdural effusion, and postoperative subdural effusion samples (table 2).

DISCUSSION

We found chronic subdural haematoma and effusion to contain extremely high concentrations of the propeptides of type I and type III procollagens, that of PICP being 78-fold and that of PIIINP 156-fold relative to the corresponding serum concentrations, and both more than 100-fold relative to concentrations that have been measured in the CSF.⁴ Interestingly, the concentrations of PICP and PIIINP were fairly similar to those observed in wound fluid during the first few days after a surgical operation,^{10–12} suggesting that dural fibroblasts have a considerable potential for collagen synthesis.

We have previously shown that the intrathecal compartment is a site for active collagen synthesis, and that procollagen propeptides increase in the CSF in a time dependent manner after SAH.^{4–7} PICP and PIIINP are already elevated on day 3 after SAH in the cisternal CSF,⁷ reach a maximum in two weeks in the lumbar CSF, and then decline slowly over several weeks.⁶ The time dependent changes in CSF procollagen propeptide concentrations are similar to those observed in collagen synthesis during wound healing and in other forms of acute fibrosis.¹³ The induction of collagen synthesis in the subdural space is probably similar to that in other tissues, but, interestingly, we found that the concentrations of the procollagen propeptides in the subdural fluids were still markedly high three months after head trauma. The fibroproliferative reaction had thus been a highly active, long lasting process.

The development of chronic subdural haematoma or effusion is often a consequence of head trauma.³ Traumatic cleavage of the DBC layer resembles a skin wound as the continuity of the tissue is severed in both cases. Interestingly, we found that the propeptide concentrations in the subdural fluid samples were close to those reported in wound fluid,^{10–11} suggesting that a fibroproliferative state resembling wound healing is involved in chronic subdural haematoma. Many cytokines and growth factors have been implicated in the pathogenesis of subdural haematoma, and recent studies have

revealed increased concentrations of inflammatory cytokines such as interleukin 6 and interleukin 8,¹⁴ and of growth factors such as vascular endothelial derived growth factor and basic fibroblast growth factor in subdural haematoma fluid.¹⁵ Inflammatory cytokines are involved in wound healing, as they attract leucocytes and mesenchymal cells to the wound site.¹³ In addition, certain fibrogenic cytokines, most notably transforming growth factor β , are released and stimulate fibroblast proliferation and collagen synthesis. The increase in the synthesis of collagen may only reflect the fibroproliferative state in the dura, but, evidently, the synthesis of other components of the extracellular matrix, including glycosaminoglycans and proteoglycans, is increased as well. The latter molecules are highly hydrophilic, and their presence in subdural fluid may increase the oncotic pressure. Indeed, not all the protein in the haematoma is derived from serum protein exudation, but part is synthesised in situ and there is an inverse relation in subdural haematoma between the total protein concentration and albumin ratio.¹⁶ The oncotic pressure of subdural fluid depends in a linear fashion on the total protein concentration.¹⁷ We therefore suggest that the increased synthesis of extracellular components leads to an increased oncotic pressure in the fluid in the cavity, thereby contributing to haematoma or effusion enlargement.

In conclusion, we found markedly high concentrations of the procollagen propeptides of type I and type III collagens in subdural fluid samples obtained within three months of head trauma, implying that the increase in dural collagen synthesis is a long lasting one. We suggest that increased synthesis of the various components of the extracellular matrix contributes to the pathogenesis of subdural haematoma and effusion.

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REFERENCES

- 1 **Frederickson RG**. The subdural space interpreted as a cellular layer of meninges. *Anat Rec* 1991;**230**:38–51.
- 2 **Haines DE**. On the question of a subdural space. *Anat Rec* 1991;**230**:3–21.
- 3 **Heines DE**, Harkey HL, Al-Mefty O. The "subdural" space: a new look at an outdated concept. *Neurosurgery* 1993;**32**:111–20.
- 4 **Sajanti J**, Majamaa K. Detection of meningeal fibrosis following subarachnoid hemorrhage by assaying procollagen propeptides in the CSF. *J Neural Neurosurg Psychiatry* 1999;**67**:185–8.
- 5 **Sajanti J**, Björkstrand AS, Finnä S, *et al*. Increase of collagen synthesis and deposition in the arachnoid and the dura following subarachnoid hemorrhage in the rat. *Biochim Biophys Acta* 1999;**1454**:209–16.
- 6 **Sajanti J**, Heikkinen E, Majamaa K. Transient increase in procollagen propeptides in the CSF after subarachnoid hemorrhage. *Neurology* 2000;**55**:359–63.
- 7 **Sajanti J**, Heikkinen E, Majamaa K. Rapid induction of meningeal collagen synthesis in the cerebral cisternal and ventricular compartments after subarachnoid hemorrhage. *Acta Neurochir (Wien)* 2001;**143**:821–6.
- 8 **Prockop DJ**, Kivirikko KI. Collagens: molecular biology, diseases, and potentials for therapy. *Annu Rev Biochem* 1995;**64**:403–34.
- 9 **Risteli J**, Risteli L. Analysing connective tissue metabolites in human serum. Biochemical, physiological and methodological aspects. *J Hepatol* 1995;**22**(suppl 2):77–81.
- 10 **Haukipuro K**, Risteli L, Kairaluoma MI, *et al*. Aminoterminal propeptide of type III procollagen in healing wound in humans. *Ann Surg* 1987;**206**:752–6.
- 11 **Haukipuro K**, Melkko J, Risteli L, *et al*. Synthesis of type I collagen in healing wounds in humans. *Ann Surg* 1991;**213**:75–80.
- 12 **Haukipuro K**, Risteli L, Kairaluoma MI, *et al*. Aminoterminal propeptide of type III procollagen in serum during wound healing in human beings. *Surgery* 1990;**107**:381–8.
- 13 **Davidson JM**. Wound repair. In: Gallin JI, Goldstein IM, Snyderman R, eds. *Inflammation: basic principles and clinical correlates*, 2nd edn. New York: Raven Press, 1992:809–19.
- 14 **Suzuki M**, Endo S, Inada K, *et al*. Inflammatory cytokines locally elevated in chronic subdural haematoma. *Acta Neurochir (Wien)* 1998;**140**:51–5.
- 15 **Weigel R**, Schilling L, Schmiedek P. Specific pattern of growth factor distribution in chronic subdural hematoma (CSH): evidence for an angiogenic disease. *Acta Neurochir (Wien)* 2001;**143**:811–18.
- 16 **Fujisawa H**, Nomura S, Tsuchida, *et al*. Serum protein exudation in chronic subdural haematoma: a mechanism for haematoma enlargement? *Acta Neurochir (Wien)* 1998;**140**:161–6.
- 17 **Weir B**. Oncotic pressure of subdural fluids. *J Neurosurg* 1980;**53**:512–15.