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Inflammatory Cytokines Locally Elevated in Chronic Subdural Haematoma

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Summary

The involvement of inflammation in the development and propagation of chronic subdural haematoma (CSH) was investigated by measuring the levels of inflammatory cytokines (tumour necrosis factor [TNF]α, interleukin [IL]-1β, IL-6, and IL-8). Peripheral venous blood and subdural fluid were obtained at the time of burr hole surgery from 34 patients with CSH and from 9 with subdural effusion. The levels of the inflammatory cytokines were analysed by enzyme-linked immunosorbent assay. The blood levels of TNFα, IL-1β, IL-6, and IL-8 in both CSH and subdural effusion groups were almost within the range of normal subjects, and no differences were observed between the two groups. IL-6 and IL-8 in the subdural fluid were much higher than in the blood of both groups, and the levels in CSH patients were significantly higher (10 times) than in subdural effusion patients. Local elevation of inflammatory cytokines in the subdural space of both CSH and subdural effusion without systemic change suggests the presence of local inflammation in the two diseases. The same behavioural patterns of cytokines for these and higher levels of cytokines in the CSH also suggest that inflammatory cytokines may be involved in the continuous development from subdural effusion to CSH and propagation of CSH.

Keywords: Chronic subdural haematoma; cytokines; subdural effusion; inflammation.

Introduction

Chronic subdural haematoma (CSH) was originally described as an inflammatory disease under the name ''pachymeningitis haemorrhagica interna'' [33]. Several indications of inflammation, such as proliferation of fibroblasts, immature capillaries, and collagen fibrils, and infiltration of inflammatory cells have been described in the outer membrane of CSH [27, 32, 38]. The causative factor of CSH is trauma, not inflammation [32]. However, the current concept of ''inflammation'' has expanded from infection or tissue repair to include head injury, cerebral infarction, subarachnoid haemorrhage, degenerative diseases, and brain tumours [11, 15, 17, 18, 23, 31, 35, 36]. Therefore, inflammatory reaction is also likely to occur in CSH following head injury.

Recently, interest has been focused on local fibrinolytic activity in the outer membrane of CSH [3, 20]. Immature capillaries in the outer membrane produce tissue type plasminogen activator, which might cause local hyperfibrinolytic activity and resultant bleeding [3]. The vasculature also has high permeability to blood components and causes enlargement of CSH [6, 37]. Immature capillaries are an ubiquitous phenomenon during angiogenesis in inflammation, and are usually accompanied by hyperfibrinolytic activity and increased permeability like that in the outer membrane [7, 8]. Therefore, CSH can be studied as a type of inflammatory phenomenon.

Neurological imaging has shown that CSH may develop following subdural effusion [13, 22], but no factor promoting CSH from subdural effusion has been identified. Inflammatory cytokines (tumour necrosis factor [TNF]α, interleukin [IL]-1β, IL-6, and IL-8) are considered to be involved in the inflammatory reaction and are used as indicators in various neurological disorders [12, 17, 18, 25, 29, 35, 36]. However, the levels of inflammatory cytokines have never been measured in the subdural fluid of patients with CSH and/or subdural effusion.

This study analysed these indicators of inflammation in the blood and subdural fluid of patients with CSH or subdural effusion in order to investigate the involvement of inflammation in the mechanism of initiation and propagation of CSH following subdural effusion.

Table 1. *Inflammatory Cytokines in Blood and Subdural Fluid**

	TNFα (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)
Normal subjects				
blood	<20	<2	<10	<3
Patients with CSH				
blood	3.5 ± 0.5	<2	4.8 ± 1.5	7.0 ± 5.6
subdural fluid	41.1 ± 8.8[a]	<2	2549 ± 395.9[b,d]	1597.6 ± 451.5[b,d]
Patients with subdural effusion				
blood	<3	<2	5.0 ± 2.7	<3
subdural fluid	7.8 ± 4.4	<2	256.3 ± 93.7[c]	129.5 ± 51.9[c]

* Values are means ± standard errors.
TNFα tumour necrosis factor-α, *IL-1β* interleukin-1β, *IL-6* interleukin-6, *IL-8* interleukin-8.
[a] $p < 0.05$, [b] $p < 0.0001$ vs. value in the blood of patients with CSH.
[c] $p < 0.05$ vs. value in the blood of patients with subdural effusion.
[d] $p < 0.0001$ vs. value in the subdural fluid of patients with subdural effusion.

Patients and Methods

Thirty-four patients, 22 males and 12 females aged from 43 to 84 years (mean 73.8 years) with CSH, and nine patients, 6 males and 3 females aged from 65 to 76 years (mean 71.1 years) with subdural effusion, were studied. The diagnoses were based on X-ray computed tomography (X-CT) and operative findings. CSH was defined as a subdural fluid collection with higher X-CT density than cerebrospinal fluid (CSF), an apparent outer membrane beneath the dura mater, and content including blood cells or their debris. Subdural effusion was defined as a liquid collection with almost the same X-CT density as CSF, a thin or no outer membrane, and clear to xanthochromic contents without blood cells. The primary cause of CSH was head injury or unknown. Subdural effusion was caused by trauma in 3 patients and intracranial surgery in 6 (patients with malignant tumour were excluded).

Samples of subdural fluid and venous blood were obtained at the time of burr hole irrigation surgery under local anaesthesia. Samples were centrifuged at 1700 g (3000 rpm) for 10 minutes immediately, and the supernatant plasma was stored in sealed plastic tubes (NALGEN® Cryogenic vials, Nalge Company, Rochester, NY) at −80 °C until analysis. Inflammatory cytokines were assayed using the following enzyme-linked immunosorbent assay kits: TNFα (MEDGENIX DIAGNOSTICA, Brussels, Belgium), IL-1β (Ohtsuka, Tokyo, Japan), IL-6 (Toray-Fuji Bionics Inc., Tokyo, Japan), IL-8 (Toray-Fuji Bionics Inc.).

CSH patients were classified into two types according to the X-CT pattern: the layering type consisting of an upper hypodense and lower hyperdense portion, which is reported to be active and recurrent [20, 26 and others]. Ten of the 34 patients were classified as the layering type.

The levels of inflammatory cytokines in the blood were compared with those in the subdural fluid in both CSH and subdural effusion patients (Wilcoxon signed-ranks test). Differences in the values in subdural fluid and in blood between the CSH and subdural effusion patients were analysed (Mann-Whitney-Wilcoxon test). The values in the subdural fluid were also compared between patients with the layering type and the others (Mann-Whitney-Wilcoxon test).

Results

The levels of cytokines in the blood of normal subjects and patients with CSH or subdural effusion are given in Table 1.

The blood from patients with CSH contained levels of TNFα, IL-1β, and IL-6 which were almost within the ranges of normal subjects. The levels of IL-8 in the blood of patients with CSH were slightly higher than those in normal subjects. The subdural fluid from patients with CSH contained the same level of IL-1β and a significantly higher level of TNFα than that in blood ($p < 0.05$). The levels of IL-6 and IL-8 in the subdural fluid were very much higher than those in blood ($p < 0.0001$).

The blood from patients with subdural effusion contained TNFα, IL-1β, IL-6, and IL-8, which were almost within the range of normal subjects. The levels of IL-6 and IL-8 were significantly higher in the subdural fluid than those in blood ($p < 0.05$).

There were no significant differences in levels of cytokines in blood between CSH and subdural effu-

Table 2. *IL-8 in the Subdural Fluid of Patients with the Layering Type and Other CSH**

	IL-8 (pg/ml)
Layering type	3258.2 ± 1138.3[a]
Others	947.9 ± 383.5

* Values are means ± standard errors.
IL-8 interleukin-8.
[a] $p < 0.05$ vs. value in others.

sion patients. However, the levels of IL-6 and IL-8 in the subdural fluid of CSH patients were significantly higher than those of subdural effusion patients ($p < 0.0001$).

The levels of IL-8 in the subdural fluid of patients with the layering type were significantly higher than those in the others ($p < 0.05$), but levels of IL-1β, TNFα, and IL-6 were not (Table 2).

Discussion

Systemic and Local Inflammation

Levels of inflammatory cytokines in the blood of CSH and subdural effusion patients were within the range of, or slightly higher than, those of normal subjects. Furthermore, there was no significant difference between the two patient groups. Therefore, the systemic inflammatory condition was basically the same in the two patient groups, and was likely to be almost the same as in normal subjects. In contrast, local levels of inflammatory cytokines in the subdural fluid, except IL-1β in both groups and TNF-α in the subdural effusion patients, were significantly higher than those in the blood in both groups. This suggests that the cytokines in the subdural fluid did not permeate from the blood but were synthesized and released *in situ* from cells facing the subdural space or from inflammatory cells infiltrating into the space, and that local and specific inflammation occurred. Inflammatory cytokines are present at high levels in the CSF of patients with several neurological diseases, supporting the idea that inflammation may be involved in the mechanism of these diseases: IL-1β, IL-6, IL-8, and TNFα in meningitis [4, 28]; IL-6 in subarachnoid haemorrhage [15]; IL-6 and TNFα in head injury [16, 25]; IL-8 in brain tumour [17]; and IL-6 in human T-cell leukemia virus-associated myelopathy [19]. The major component of subdural fluid is CSF in cases of subdural effusion, and the situation is similar to meningitis or subarachnoid haemorrhage where the main lesions are on the brain surface without massive brain damage. Levels of IL-6 and IL-8 were also elevated in our subdural effusion cases, suggesting the presence of common inflammatory factors.

However, comparison of our data from subdural fluid in CSH and previous data obtained from CSF may be erroneous, as the subdural fluid in CSH is encapsulated by the outer and inner membranes, and separated from the central nervous tissue and CSF. The only valid comparison of cytokines in the subdural fluid of patients with CSH may be with patients with subdural effusion.

Origins of Inflammatory Cytokines

Inflammatory cytokines present in the central nervous tissue affected by neurological diseases are secreted by astrocytes, microglia, or infiltrating inflammatory cells [2, 10, 24]. However, the outer and inner membranes of CSH are likely to prevent permeation from the CSF, so other sources of cytokines than astrocytes or microglia must be considered. The membrane of CSH consists of fibroblasts, endothelial cells, and inflammatory cells, which are generally known to produce numerous different cytokines, and are likely to secrete IL-6 and IL-8. Many stimulators of inflammation such as thrombin or platelet activating factor are released after bleeding [1, 5]. Therefore, these cells are likely candidates to secrete cytokines into subdural fluid as a response to bleeding [30, 34].

Inflammatory Mechanism of CSH Formation

Recently, neuro-imaging has revealed that CSH may develop following subdural effusion [13, 22]. Presumably an outer membrane is already formed even at the stage of subdural effusion. Indeed, five of 26 patients with subdural effusion operated on more than 3 weeks after initial injury had an outer membrane [21]. This immature outer membrane may be the source of the cytokines in subdural effusion. The same behavioural pattern of IL-6 and IL-8 in the subdural fluid of both CSH and subdural effusion, and lower levels of the two cytokines in the subdural effusion, suggest the presence of a common background of local inflammation in the two diseases, and supports the possibility of continuous development from subdural effusion to CSH. Interestingly, IL-6 causes enlargement of the gap between endothelial cells and increases vascular permeability [14]. Such a gap widening was also observed in the outer membrane of CSH [27, 37]. Furthermore, IL-8 is a potent angiogenetic factor [9], and may contribute to the growth of immature capillaries with fibrinolytic activity in the outer membrane. This function may explain our observation that IL-8 was significantly elevated in the subdural fluid in the layering type CSH, which is considered as active and easy to rebleed [20, 25]. These considerations suggest that suppression of local inflammatory cytokines in the subdural space may reduce the risk of rebleeding or propagation of CSH,

and prevent development of CSH from subdural effusion.

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Comment

This is an excellent study on a very common but complex neurological disease: chronic subdural haematoma. The aim of the study is original, the methodology is sound, the results are unequivocal and the discussion is to the point.

The separation between chronic subdural haematoma and subdural effusion may be somewhat artificial and arbitrary, but these entities have been well defined. It was stated and supported by literature references, that chronic subdural haematoma develops from subdural effusion, but this does not exclude the possibility of chronic subdural haematoma developing "de novo" without preceding subdural effusion. In the section on clinical materials and methods the control group of normal patients was not mentioned i.e. the number of normals and whether this group was matched for example with regard to age.

The findings of this study contribute to a better understanding of the pathogenesis of this condition and, furthermore, may lead to new treatment modalities.

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