Possible role of cyclooxygenase-2 in developing chronic subdural hematoma

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Inflammatory cytokines are reportedly involved in the pathogenesis of chronic subdural hematomas (CSH), and the angiogenesis of hematomas has particularly been in focus. Cyclooxygenase-2 (COX-2) is an essential enzyme for the synthesis of prostaglandin E\textsubscript{2} (PGE\textsubscript{2}). The COX-2-PGE\textsubscript{2} pathway has been shown to influence angiogenic factors such as vascular endothelial growth factor (VEGF). We investigated the association of COX-2 expression in the dura mater and outer membrane with the pathogenesis of CSH, and suggested a treatment strategy on the basis of this association. Hematoma fluid and serum samples obtained from 37 patients, and samples of the dura mater and outer CSH membrane obtained from 13 patients during the operation were examined in this study. The concentrations of PGE\textsubscript{2} in relation to COX-2 in the hematoma fluid were significantly higher than those in the serum. Immunohistochemical analyses revealed COX-2-positive cells in the outer membrane of CSHs. There was a linear and significant relationship between PGE\textsubscript{2} concentration in hematoma fluid and the interval from trauma to initial surgery. COX-2 may play a crucial role during the development of CSHs. Our study might lead to the development of anti-COX-2 treatment options that aim to minimize repeat surgery and choose medical therapy by reducing CSH morbidity and recurrence rate in patients with CSH.

Key words: chronic subdural hematoma, cyclooxygenase-2, prostaglandin E\textsubscript{2}.

Introduction

Chronic subdural hematoma (CSH) is one of the most common diseases affecting the aged, though it sometimes involves younger individuals, including children. CSH develops as an encapsulated hematoma in the subdural space 2 weeks or more after minor head injuries. The clinical course of this disease is well known, but its causative mechanism remains unclear. Virchow described CSH as an inflammatory disease and named it pachymeningitis haemorrhagica interna. Later, several studies demonstrated that this process is a local inflammatory reaction of the dura mater in response to external stimuli such as trauma, blood, cerebrospinal fluid (CSF), fibrin, or fibrin degradation products. In fact, as mesenchymal cells in the dural border proliferate and differentiate, they form a sort of inflammatory capsule or membrane around the blood clots or CSF, called the external or outer membrane of the CSH. The outer membrane of a CSH is composed of granulation-like tissue, which also contains immature vessels and connective fibers, and on the whole, constitutes a source of inflammatory, angiogenic, fibrinolytic, and coagulation factors.

The pathological vascularization of this outer membrane is thought to play a crucial role in the pathogenesis of CSH. Recently, evidence in favor of marked alterations in the local expression of important angiogenic factors has been presented. The observations include (1) a suspiciously high concentration of VEGF in hematoma fluid and (2) stimulation of VEGF gene expression in cells freely floating in hematoma fluid. Cianchi et al. identified a significant correlation between COX-2 and VEGF; the latter might be one of the most important mediators of the COX-2 angiogenic pathway. PGE\textsubscript{2}, whose synthesis from arachidonic acid is catalyzed by COX-2, regulates VEGF expression, and COX-2 inhibitors directly affect angiogenesis. If COX-2 is overexpressed in the outer membrane of CSH...
and the hematoma fluid has a high concentration of VEGF and PGE₂ in relation to COX-2. COX-2 inhibitors may be a new therapeutic modality for CSH. However, there has been no report on the role of COX-2 in CSH development.

In the present study, we investigated the concentrations of interleukin (IL)-6, IL-8, VEGF, and PGE₂ in the hematoma fluid and serum from CSH patients.

**Materials and Methods**

**Patients**

We examined 37 patients with CSH, of whom 25 were male and 12 female, at the Tokyo Medical and Dental University Hospital between February 2003 and March 2004. The ages of the patients ranged from 26 to 94 years (mean, 71.4 ± 13.6 years), and CSH was diagnosed on the basis of computed tomography (CT). Of the 37 patients, 16 had experienced apparent head trauma in the past. The patients had not received any previous treatment for CSH. We treated all the patients surgically by a small burr-hole craniotomy and irrigation under local anesthesia, followed by closed external drainage for 1 night. Hematoma fluid and serum samples were obtained from all the patients, and samples of the dura mater and outer membrane were obtained from 13 patients during their operation (Fig. 1). The hematoma fluid and serum samples were centrifuged at 3000 rpm for 10 min immediately after the operation, and the supernatant was stored at −80°C until assayed. Indomethacin was added to the samples of hematoma fluid and serum immediately after sample collection to block prostaglandin synthesis. We obtained written informed consent for the analyses of the materials from individual patients or their families. The protocol of this study was approved by the institutional ethics committee of Tokyo Medical and Dental University.

**Measurement of IL-6, IL-8, VEGF, and PGE₂**

We measured the concentrations of IL-6, IL-8, VEGF, and PGE₂ in hematoma fluid and serum by using enzyme-linked immunosorbent assay (ELISA) kits for human IL-6, IL-8, VEGF (TECHNE Corporation, Minneapolis, MN), and PGE₂ (Cayman Chemical Company, Ann Arbor, MI). Furthermore, we measured the concentrations of IL-6, IL-8, VEGF, and PGE₂ in CSF obtained from 4 patients with hydrocephalus. The normal concentrations of IL-6, IL-8, VEGF, and PGE₂ in serum were cited from previous reports.

**Immunohistochemistry**

The overlying dura and outer membrane of CSH were obtained during primary burr hole surgery. Resected specimens were fixed in 10% formalin solution, routinely processed, embedded in paraffin and sectioned at a thickness of 7 μm. These sections were autoclaved at 121°C for 20 min in 10 mM citrate buffer at pH 6.0. The sections were immersed in 3% H₂O₂, prepared in distilled water, for 20 min and then in rabbit serum for 30 min to block endogenous peroxidase activity and the nonspecific binding sites, respectively. Goat polyclonal antibody for human COX-2 (Santa Cruz, CA, USA) diluted 1:80 was then added to the sections, and they were incubated overnight at 4°C. After washing with Tris-buffered saline, a secondary biotin-labeled rabbit anti-goat antibody (IgG) diluted 1:400 was added to the sections, and they were incubated for 30 min. The sections were also incubated with a monoclonal antibody against human macrophage CD68 (DAKO, Glostrup, Denmark), and were then incubated with biotinylated secondary anti-mouse antibodies. Immunohistochemical detection was carried out using the labeled streptavidin-biotin method (DAKO). The sections were finally developed with diaminobenzidine and counterstained with hematoxylin.

**Analysis of PGE₂ and the interval from trauma to initial surgery**

In the 16 patients who had previously experienced head trauma, we analyzed the correlation between the interval from trauma to initial surgery and the PGE₂ concentration in the hematoma fluid.
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Statistical Analysis

All values are expressed as mean ± SD. Statistical significance was analyzed using a nonparametric (Kruskal-Wallis) test. The statistical significance between the trauma group and non-trauma group was analyzed using Student’s t-test. The correlation between the interval from trauma to initial surgery and PGE2 concentration in the hematoma fluid was analyzed using Pearson’s correlation test. P < 0.01 was considered to be statistically significant.

Results

The concentrations of IL-6, IL-8, VEGF, and PGE2 in the hematoma fluid were markedly elevated. The concentrations of IL-6 and VEGF in the serum samples were almost within the normal range. The concentrations of IL-8 in serum were slightly higher than those in normal subjects. The concentrations of IL-6, IL-8, VEGF, and PGE2 in the hematoma fluid were significantly higher than those in serum (Fig.2).

Hematoxylin-eosin (HE) staining of the outer membrane showed vascularized and fibrocollagenous tissue infiltrated by inflammatory cells, such as neutrophils, eosinophils, lymphocytes, macrophages, and plasma cells (Fig.3A). The endothelial cells of sinusoids and capillaries and the inflammatory cells in the outer membrane were observed to be positive for COX-2 (Fig.3B). The dura mater was not immunoreactive for COX-2. CD68-positive cells were present in the outer membrane, suggesting that many of the inflammatory cells are of macrophage origin (Fig.3C).

A correlation was observed between the PGE2 concentration in the hematoma fluid and the interval from trauma to initial surgery. The PGE2 level was significantly higher in the hematoma fluid than in the CSF and serum (Fig.2), whereas there was no significant difference between the PGE2 concentration in the samples of hematoma fluid obtained from the trauma group and those obtained from the non-trauma group. In the 16 patients who had previously experienced head trauma, a strong positive correlation was observed between the PGE2 concentration in the hematoma fluid and the interval from trauma to initial surgery (Fig.4). On the other hand, no such relationship was observed between the VEGF concentration and the interval from trauma to initial surgery.

Discussion

Currently, it is considered that a CSH is a chronic self-perpetuating inflammatory process that involves the dura mater, and develops in response to injury or external stimuli such as trauma, blood, CSF, fibrin, or fibrin degradation products. Several indications of inflammation, such as proliferation of fibroblasts, immature capillaries, and collagen fibrils, and infiltration by inflammatory cells have been described in the outer membrane of a CSH. Research interest has been focused on the local activity in the outer membrane of a CSH.
This outer membrane is a source of tissue plasminogen activator and inflammatory cytokines. Therefore, CSH can be studied as a type of inflammatory phenomenon. The pathological analyses of the outer membrane of a CSH strongly suggest ongoing angiogenesis. Suzuki et al. first described the higher VEGF concentrations in hematomas than in the serum. Shono et al. also reported the higher concentrations of VEGF in the hematoma fluid than in serum, and an increased expression of VEGF and macrophages in the outer membrane of CSHs. By using immunohistochemical staining for VEGF, Vaquero et al. found the source of angiogenic factors to be the granulation tissues (outer membrane) of the CSH. Neovascularization with vascular hyperpermeability in the outer membrane has been identified in surgical specimens, and VEGF is deemed pathognomonic for this structural integrity, although there is still some discordance on this issue.

In this study, we found numerous CD68-positive macrophages in the outer membrane. A variety of cytokines are secreted from these immune cells and macrophages. Suzuki et al. reported the local elevation of inflammatory cytokines, such as IL-6 and IL-8, in the subdural space of CSH and subdural effusion. These cytokines may be responsible for initiating COX-2 expression. In the present study, we found the overexpression of COX-2 in the outer membrane of CSH, and elevated levels of PGE2 in the hematoma fluid. The COX-2-PGE2 pathway has been shown to influence angiogenic factors such as VEGF. An increased expression level of COX-2 in the outer membrane of CSH would lead to an increase in prostaglandin production. It is thought that the major role of COX-2 in angiogenesis is the triggering of the synthesis of prostanoids such as PGE2.
then stimulate the expression of VEGF.\textsuperscript{26}

The overexpression of COX-2 and the accompanying increase in prostaglandin production likely create an abnormal state in the outer membrane. The production of prostaglandins plays an important role in regulating the production of angiogenic factors\textsuperscript{26,30} and increasing vascular permeability. The overexpression of COX-2 in human colon carcinoma cells results in angiogenesis by factors such as VEGF.\textsuperscript{16,27} Matsumori et al. demonstrated that prostaglandin participates in both the formation and healing of CSHs.\textsuperscript{19} On the other hand, Katano et al. showed a hypothetical time course for development of CSH with a gradual surge in VEGF levels.\textsuperscript{30} Our study showed that there is a positive correlation between the concentration of PGE\textsubscript{2} in the hematoma fluid and the interval from trauma to initial surgery, whereas this correlation was not observed in case of VEGF. The changes of PGE\textsubscript{2} concentrations in the hematoma fluid are probably associated with the development of CSH. The high levels of PGE\textsubscript{2} and VEGF or vascular permeability factor in the hematoma fluid might influence the functional status of vessels inside the outer membrane, causing leakage and extravasation of proteins, and this is an important driving force for hematoma enlargement. VEGF concentrations in the hematoma fluid vary widely, and there was no positive correlation with the interval from trauma to initial surgery because VEGF is produced via several pathways. However, PGE\textsubscript{2} is one of promoters of VEGF and produced via the COX-2-PGE\textsubscript{2} pathway.

The widespread availability of cross-sectional imaging has markedly increased the number of asymptomatic CSH patients or patients in whom the CSH is still developing; such patients do not need immediate evacuation of the hematoma, and can be placed under observation. Our data suggest that drug-based treatment strategies such as the administration of COX-2 inhibitors might provide new and promising pharmacological alternatives to inhibit the growth of CSH, presumably by interfering with the COX-2 angiogenic pathway. Such treatment might even supersede neurosurgical intervention in CSH patients, and potentially decrease the rate of morbidity and mortality of this disease.

In the present study, we showed that there was a linear and significant correlation between PGE\textsubscript{2} concentration in hematoma fluid and the interval from trauma to initial surgery. High PGE\textsubscript{2} concentrations are thought to reflect an increased activity of COX-2. Thus, in conclusion, COX-2 may play a crucial role in the development of CSH.

References


