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Opinion paper

Role of cathepsin K in the development of chronic subdural hematoma

Satoshi Tsutsumi [a,*], Ikuko Ogino [b], Masakazu Miyajima [b], Senshu Nonaka [a], Masanori Ito [a], Yukimasa Yasumoto [a], Hajime Arai [b]

[a] Department of Neurological Surgery, Juntendo University Urayasu Hospital, Japan
[b] Department of Neurological Surgery, Juntendo University School of Medicine, Japan

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ABSTRACT

Despite extensive investigations, the process of development of chronic subdural hematoma (CSDH) is not known. The present study aims to investigate CSDH by measuring biomarkers in it, gas analysis, and immunohistochemical examination. A total of 42 patients with symptomatic CSDH who underwent burr-hole drainage were enrolled. Intraoperatively, hematoma fluid and peripheral venous blood (PV_{CSDH}) were simultaneously collected. As controls, peripheral venous blood ($PV_{Control}$) and intracranial cerebrospinal fluid (CSF) were collected from other subjects during other surgeries. CatK, lipocalin-type prostaglandin D synthase (PGDS), and cystatin C (CysC) present in these specimens were measured using enzyme-linked immunosorbent assay. Data obtained were statistically analyzed after age correction. In 15 patients, gas analysis was performed for CSDH and PV_{CSDH}. Furthermore, immunohistochemical examination for the outer membrane was performed for four patients. CatK, PGDS, and CysC levels were markedly elevated in the CSF and CSDH. CatK levels in PV_{CSDH} were significantly higher than in $PV_{Control}$ ($P < 0.0001$). In contrast, CysC levels in PV_{CSDH} were significantly lower than in $PV_{Control}$ ($P = 0.004$). The gas analysis revealed that the internal environment of CSDH is characterized by marked hypoxia, hypoglycemia, and lactic acidosis. Furthermore, the outer membrane consistently showed a diffuse staining for CatK. Based on these, CatK was thought to play a role in the development of CSDH, with the levels in peripheral venous blood elevated in patients with CSDH.

1. Introduction

Cathepsin is a lysosomal protease with 15 subtypes identified to date. Cathepsin K (CatK), a member of the cathepsin family, was initially found to be present on osteoclasts and was thought to be associated with the pathogenesis of osteoporosis and autoimmune arthritis [1–3]. Recently, CatK was documented to activate Notch1 and its downstream cascades, including vascular endothelial growth factor (VEGF), in response to hypoxia, which consequently promotes neovascularization [4,5]. Furthermore, it has been suggested that CatK may be associated with local inflammation in periodontitis and rheumatoid arthritis [6,7]. Odanacatib, a CatK antagonist, has been under development as a novel therapeutic agent of osteoporosis [8]. However, an increased risk for stroke was proven during the trial and the trial was discontinued.

The normal CatK level in human peripheral venous blood and cerebrospinal fluid (CSF) has not yet been reported.

Chronic subdural hematoma (CSDH) is a frequent clinical entity and commonly acknowledged as a slow growing fluid hematoma, capsulized by the inner and outer membranes. It is thought to become symptomatic weeks to months after a minor head trauma. Despite extensive investigations; however, its etiology is not known. Instead, there have been a number of studies documenting fragmentary findings associated with CSDH. Advanced age, male sex, subdural CSF leakage following head trauma, subdural hygroma, and surgeries for cerebral aneurysms requiring extensive arachnoid dissection have been proposed as contributory factors in CSDH development [9–12].

Mechanisms underlying the development of the inner and outer membranes of CSDH are also elusive. Watanabe et al. reported in an experimental study that membranous fibrin that forms on the surface of the clot, in the presence of CSF, was important for the development of the outer membrane [13]. However, no further studies have explored this phenomenon. In a clinical setting, magnetic resonance (MR) angiography and contrast MR imaging have been proposed to be useful for evaluating the maturation of the outer membrane and enlargement of the CSDH [14,15].

* Corresponding author at: Department of Neurological Surgery, Juntendo University Urayasu Hospital, 2-1-1 Tomioka, Urayasu, Chiba 279-0021, Japan.
E-mail address: shotaro@juntendo-urayasu.jp (S. Tsutsumi).

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Since initial documentation of excessively activated coagulation and fibrinolysis in the hematoma fluid [16], many biomarkers associated with CSDH have been proposed. These include platelet-activating factor (PAF), angiopoietin-1 and -2, matrix metalloproteinase (MMP)-2 and -9, placental growth factor (PlGF), VEGF, transforming growth factor (TGF)-β, activin receptor-like kinase 1 (ALK-1), and interleukin (IL)-6 and -8 [17–22].

Histologically, the outer membrane responsible for the growth of a CSDH is characterized by proliferation and excessive thickness of the dural border cell layer, accompanied by formation of capillaries and collagen fibrils [23].

Commonly, burr-hole drainage with variable modifications has been used as a treatment for symptomatic CSDH. However, there is no clear evidence supporting the superiority of specific maneuvers [24,25]. On the other hand, the efficacy of steroid therapy for low-grade CSDH and selective middle meningeal artery embolization for refractory recurrent cases have been documented [26,27]. Recently, an inhibitory mechanism targeting the growth of the outer membrane has been proposed as a potential therapy [28].

The present study aimed to investigate CSDH by measuring levels of CatK and CSF-specific biomarkers, lipocalin-type prostaglandin D synthase (PGDS) [29] and cystatin C (CysC) [30] that are present in CSDH and peripheral venous blood (PV_{CSDH}), gas analysis of CSDH and PV_{CSDH}, and by immunohistochemical examination of the outer membrane.

2. Methods

This prospective study was performed in accordance with the guidelines for human research of the institution. Written informed consent was obtained from all patients.

The study included 42 adult patients with symptomatic CSDH who presented to the hospital with gait disturbance, hemiparesis, impaired consciousness, and headache, between July 2014 and August 2016. These patients provided 49 sides of CSDH in total. The 42 patients comprised 34 men and 8 women, with the mean age of 75.1 ± 10.9 years. Diagnosis of symptomatic CSDH was made on the patients' neurological findings and appearance on plain computed tomography (CT) scans at presentation. Symptomatic subdural fluid collection appearing with a homogenous low density on CT scans was diagnosed as subdural hygroma and was excluded from the study. Patients with disorders of the respiratory and cardiovascular system were also excluded.

For all 42, single burr-hole drainage was performed using local anesthetics, under sedation with intravenous midazolam infusion. Moreover, pure oxygen was provided at 3 L/min for all patients, by means of mask administration. Intraoperatively, CSDH and PV_{CSDH} in the brachial vein were simultaneously collected. CSDH fluid was collected by puncturing the outer membrane with the barrel of an indwelling needle. All samples were aspirated in 5-mL syringes and placed into test tubes without any mounting reagent, and was immediately centrifuged to remove cells and debris. The plasma fraction was aliquoted and finally stored in polypropylene tubes at −80 °C until ready for biochemical analysis [31]. CatK, PGDS, and CysC levels were measured in all samples by enzyme-linked immunosorbent assay (ELISA).

For control subjects, samples of peripheral venous blood ($PV_{Control}$) and intracranial CSF were used. The former were collected from 83 patients who underwent craniotomies at our institution, between December 2012 and January 2016, for various pathologies other than CSDH and inflammatory diseases. The latter were obtained intraoperatively from 10 of the 83 patients. These samples were collected, processed, and cryopreserved in the same way as the CSDH and PV_{CSDH} samples. Levels of CatK, PGDS, and CysC in them were also measured by ELISA.

In 15 of the 42 patients, gas analysis was performed, involving 17 sides of CSDH in total. The patient population included 13 men and 2 women, with the mean age of 73.5 ± 10.9 years. Aliquots of the intraoperatively collected CSDH and PV_{CSDH} samples were promptly placed, without bubble contamination, into respective 1-mL test tubes for gas analysis, which was performed on the same machine (RAPIDLAB®1265, Siemens, Munich, Germany). Then, the values of oxygen partial pressure (PO_2), carbon dioxide partial pressure (PCO_2), hydrogen ion exponent value (PH), glucose (Glu), lactate (Lac), Na^+, and K^+ were recorded.

Furthermore, in 4 patients, a small fragment of the outer membrane of the CSDH was resected during surgery, and CatK expression was examined using anti-CatK antibodies (Anti-Cathepsin K antibody ab19027, abcom®, Cambridge, UK). As a control, a fragment of the frontal dura mater, 5 mm × 5 mm in dimension, was harvested from 4 patients suffering from traumatic acute subdural hemorrhage and who underwent craniotomy at our institution.

2.1. Statistical analysis

The data obtained were recorded as mean value ± standard deviation (SD). The age range was corrected and set at 60–80 years before statistical analysis. The number of samples evaluated was 25 for CSDH, 27 for PV_{CSDH}, 33 for $PV_{Control}$, and 4 for CSF. Data were analyzed using the Mann–Whitney *U* test in SPSS v.18 for Windows (SPSS, Cary, NC, USA). In each population, the upper and lower limits of analysis were set at the 95% confidence interval. For all analyses, $P < 0.05$ was considered significant.

3. Results

All 25 CSDH specimens were initially collected as fluid hematomas with a typical appearance of it. There was no considerable time delay from collection to gas analysis, which may have affected values in the CSDH or PV_{CSDH} samples.

3.1. CatK, PGSD, and CysC levels in CSDH, $PV_{Control}$, PV_{CSDH}, and CSF

3.1.1. CatK level

CatK levels in CSDH, $PV_{Control}$, PV_{CSDH}, and CSF were 31.7 ± 25.5 pmol/ml (21.1–42.2 pmol/mL), 2.1 ± 2.0 pmol/mL (1.4–2.9 pmol/mL), 9.4 ± 7.1 pmol/mL (6.6–12.2 pmol/mL), and 33.8 ± 13.4 pmol/mL (12.5–55.0 pmol/mL), respectively. CatK levels were markedly elevated in CSF and CSDH fluid, without significant differences between them ($P = 1.0$). Compared to CSDH, the CatK level was significantly lower in $PV_{Control}$ ($P < 0.0001$) and PV_{CSDH} ($P = 0.001$) samples. Furthermore, compared to $PV_{Control}$, CatK levels were significantly higher in PV_{CSDH} ($P < 0.0001$; Fig. 1).

3.1.2. PGDS level

PGDS levels in CSDH, $PV_{Control}$, PV_{CSDH}, and CSF were 1967.1 ± 758.2 ng/mL (1667.2–2267.1 ng/mL), 202.8 ± 159.3 ng/mL (146.3–259.3 ng/mL), 259.8 ± 149.0 ng/mL (200.9–318.8 ng/mL), and 6174.0 ± 1504.0 ng/mL (3780.8–8567.2 ng/mL), respectively. PGDS levels were markedly increased in CSF and CSDH samples, and the level in CSF was significantly higher than in CSDH samples ($P = 0.037$). Compared to CSDH, PGDS levels were significantly lower in $PV_{Control}$ and PV_{CSDH} samples ($P < 0.0001$). Although PGDS levels in PV_{CSDH} tended to be higher than in $PV_{Control}$, the difference did not reach statistical significance ($P = 0.63$; Fig. 2a).

3.1.3. CysC level

CysC levels in CSDH, $PV_{Control}$, PV_{CSDH}, and CSF were 600.0 ± 263.3 μg/L (488.8–711.3 μg/L), 423.8 ± 197.2 μg/L (353.9–493.7 μg/L), 265.3 ± 143.1 μg/L (208.7–321.9 μg/L), and

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