Changes in the Level of 7 α -Hydroxy-3-oxo-4-cholestenoic Acid in Cerebrospinal Fluid after Subarachnoid Hemorrhage

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Abstract

A high concentration of a type of cholic acid, 7 α -hydroxy-3-oxo-4-cholestenoic acid, is observed in the content of chronic subdural hematoma. To investigate the possible causes, the level of this compound was measured in the cerebrospinal fluid of patients who underwent surgery for aneurysmal subarachnoid hemorrhage or non-hemorrhagic diseases. The maximum level was significantly higher in the aneurysmal subarachnoid hemorrhage patients, indicating that surgical intervention did not cause the postoperative increase in the level of this compound in the cerebrospinal fluid. Monitoring of plasma levels showed no postoperative increase. *In vitro* culture of a mixture of arterial blood and cerebrospinal fluid failed to show the *de novo* production of this compound. These results strongly suggest extrahepatic intracranial production of this cholic acid occurs in subarachnoid hemorrhage. The high concentration of this compound in both chronic subdural hematoma and subarachnoid hemorrhage suggests a possible role for 7 α -hydroxy-3-oxo-4-cholestenoic acid in intracranial hemorrhagic disorders.

Key words: 7 α -hydroxy-3-oxo-4-cholestenoic acid, subarachnoid hemorrhage, chronic subdural hematoma, cerebrospinal fluid, intracranial hemorrhage, cholic acid

Introduction

7 α -Hydroxy-3-oxo-4-cholestenoic acid was first identified in normal human plasma in 1988.³⁾ The chemical structure (Fig. 1) was based only on gas chromatography-mass spectrometry analyses. This compound, a type of cholic acid, is not known to exist in the common pathway from cholesterol to usual cholic acids such as cholic acid itself or chenodeoxycholic acid.⁴⁾ Furthermore, this compound is not detected in bile, but is always present in normal human plasma.^{1,2)}

We previously purified and identified 7 α -hydroxy-3-oxo-4-cholestenoic acid from the contents of chronic subdural hematoma, and confirmed the structure by nuclear magnetic resonance spectroscopy and comparison with a chemically synthesized compound.⁸⁾ We found that the concentration in chronic

Fig. 1 Chemical structure of 7 α -hydroxy-3-oxo-4-cholestenoic acid.

subdural hematoma was 658.09 ± 599.48 ng/ml (mean $\pm$ SD), in contrast to that in normal human plasma of 126.27 ± 58.80 ng/ml. The biological activity is still unknown, but the clinical observations that this substance is specific to chronic subdural hematoma and is never found in subdural hygroma, and that the level in chronic subdural hematoma in-

creases five-fold to twenty-fold over the normal plasma level, suggest that this substance may be important in the pathogenesis of chronic subdural hematoma.⁷⁾

This study investigated several possible causes of the high level of 7 α -hydroxy-3-oxo-4-cholestenic acid in cerebrospinal fluid (CSF) after aneurysmal subarachnoid hemorrhage (SAH), to clarify the role of this substance in SAH and chronic subdural hematoma.

Materials and Methods

I. Analytical procedure

7 α -Hydroxy-3-oxo-4-cholestenic acid was incidentally found during the biochemical analysis of lipooxygenase products,¹⁰⁾ especially hydroxy-eicosatetraenoic acids (HETEs).¹²⁾ Therefore, the method of extraction is the same as that of HETEs reported by Powell.⁹⁾ Sample (1 ml) was mixed with 3 ml of ice-cold methanol and 0.5 nmol of 13-hydroxy-octadecadienoic acid as an internal standard. Distilled water was added to give a final concentration of 15% methanol. The mixture was then centrifuged at 800g for 20 minutes. The supernatant was acidified to a pH of 2.5 and passed through an octadecylsilyl (ODS) silica cartridge, Sep-Pak C₁₈ cartridge (Sep-Pak Waters Asspcoaters Inc., Milford, Mass., U.S.A.) using a glass syringe. The cartridge was washed with 10 ml of 15% aqueous methanol and then with 10 ml of n-hexane. This compound together with other polar lipids (HETE *etc.*) were eluted from ODS silica with 10 ml of ethyl acetate.^{8,12)} The solvent was evaporated under a stream of N₂. The residue was dissolved in methanol/water/acetic acid (75:25:0.01 vol/vol/vol), and subjected to reverse-phase high-performance liquid chromatography (HPLC) using a Shimadzu SLC-6A (system controller), SPD-6A (UV detector), and C-R3A Chromatopac (data analyzer) (Shimadzu, Kyoto), housing an ODS-silica column (4.6 $\times$ 150 mm) (TOSOH TSK-ODS-80TM; TOSOH, Tokyo). The mobile phase was the same solvent mixture. Flow rate was 1.2 ml/min and the chromatogram was monitored at 240 nm, which is the absorption maximum of this substance. The amount of the compound was measured from the peak area on the HPLC using a molar extinction coefficient of 13,200 M⁻¹cm⁻¹ at 240 nm, and corrected for the recovery using the peak area of the internal standard.

II. Levels of 7 α -hydroxy-3-oxo-4-cholestenic acid in normal human plasma and CSF

Plasma samples were taken from 11 normal volunteers (age 20–63 yrs), including six males and five females. CSF samples taken from four patients who underwent myelography for a cervical or lumbar disc disease were used as a normal control.

III. Time course of levels of 7 α -hydroxy-3-oxo-4-cholestenic acid in CSF and plasma after SAH

Six patients with aneurysmal SAH, who underwent craniotomy within 24 hours of the onset, were selected for this study. Continuous cisternal drainage from the prechiasmatic cistern was carried out for 10 days after surgery. The drained CSF mixed with blood was collected every 24 hours, and the levels of 7 α -hydroxy-3-oxo-4-cholestenic acid were measured. In one of these patients, the change in the plasma level was also examined.

IV. Effect of surgical intervention on the levels of 7 α -hydroxy-3-oxo-4-cholestenic acid

Postoperative concentrations in CSF were measured in four patients who underwent craniotomy for non-hemorrhagic diseases: three cases of non-ruptured aneurysms and one case of an arachnoid cyst located on the left temporal tip. The sites and sizes of the craniotomies were well matched to those in the cases of ruptured aneurysms. Cisternal drainage was placed at the prechiasmatic cistern for 24 hours, and the collected CSF was examined using the same method.

V. *In vitro* assessment of the interaction of blood and CSF

The coexistence of blood and CSF in the cranial cavity in SAH and chronic subdural hematoma was modeled by an *in vitro* culture study. Non-heparinized arterial blood (1 ml) was aseptically injected *in vitro* into 1 ml of CSF from a patient who underwent myelography for a non-neurological disorder. The mixture of blood and CSF was incubated at 37°C for 4 days. A small sample was taken from the mixture every day, and the levels of 7 α -hydroxy-3-oxo-4-cholestenic acid in each sample were measured. Every examination was performed in triplicate.

Results

I. Levels of 7 α -hydroxy-3-oxo-4-cholestenic acid in normal human plasma and CSF

The level of 7 α -hydroxy-3-oxo-4-cholestenic acid in normal human plasma was 126.27 $\pm$ 58.80 ng/ml (mean $\pm$ SD). However, this compound could not

Fig. 2 Changes with time in the level of 7 α -hydroxy-3-oxo-4-cholestenoic acid in CSF after onset of SAH. Error bars show mean $\pm$ SD. Slashed area shows the range of the mean $\pm$ 2 SD in normal plasma obtained from 11 normal volunteers.

be detected in normal CSF.

II. Time course of levels of 7 α -hydroxy-3-oxo-4-cholestenoic acid in CSF and plasma after SAH

The changes with time in the levels of 7 α -hydroxy-3-oxo-4-cholestenoic acid in CSF after SAH are shown in Fig. 2. The concentration on the day after surgery was 597.19 ± 173.03 ng/ml (mean $\pm$ SD), about five-fold the normal serum level, and gradually decreased afterwards. Among the six patients examined, two showed a second increase of the level 1 week after onset, when delayed cerebral vasospasm commonly starts. However, no definite correlation between the time course and symptomatic vasospasm was observed.

The plasma level on the day after the operation was 202.50 ng/ml which was within the normal mean $\pm$ 2 SD, and gradually decreased to 61.00 ng/ml on day 7.

III. Effect of surgical intervention on the levels of 7 α -hydroxy-3-oxo-4-cholestenoic acid

Since the increase in the level was most prominent postoperatively, and for ethical reasons, the CSF was collected only during 24-hour period after surgery in the non-SAH patients, and the cisternal drain was then removed. The concentration in this group was 193.12 ± 70.72 ng/ml (mean $\pm$ SD), which was significantly lower compared with that in the SAH group (Welch's *t*-test, $p < 0.01$).

IV. Interaction of blood and CSF *in vitro*

In the mixture of non-heparinized arterial blood and CSF of the same subject cultured for 4 days, the level of 7 α -hydroxy-3-oxo-4-cholestenoic acid was 31.67 ± 4.22 ng/ml (mean $\pm$ SD) on day 1, 37.21 ± 3.83 ng/ml on day 2, 38.21 ± 2.83 ng/ml on day 3, and 35.29 ± 3.67 ng/ml on day 4, respectively. No definite change was observed in this period.

Discussion

This study revealed that the levels of 7 α -hydroxy-3-oxo-4-cholestenoic acid in CSF after aneurysmal SAH increased five-fold over the normal plasma level. SAH is a pathological condition caused by rapid injection of arterial blood into the subarachnoid space and mixing with CSF, for example due to the rupture of a cerebral aneurysm. Since this compound is not present in normal CSF, the blood would be diluted by CSF, leading to an expected reduction of the level in the mixture of blood and CSF. The actual clinical observation was perplexing and even paradoxical. The following four mechanisms can be considered as the causes of the high level: 1) an effect of the surgical procedure; 2) an unknown interaction between blood and CSF; 3) the possible systemic elevation of the plasma level after the onset of SAH; and 4) the local, extrahepatic production of this compound. Each possibility will be further discussed below.

The effect of the surgical procedure was examined in the CSF of patients who underwent similar cranial surgery for non-hemorrhagic diseases or ruptured aneurysms. This study revealed significantly higher levels after surgery for aneurysmal SAH, although an increase in the level of this compound was seen in the other patients probably due to blood contamination of the CSF. Accordingly, the high level in CSF containing blood could not be explained simply by the surgical effect. The interaction between blood and CSF was examined in an *in vitro* study. Our experiment showed that no additional production was observed in the simple mixture of arterial blood and CSF *in vitro*. The elevation of systemic plasma level was examined by comparing the levels in blood and CSF in one of our six patients. While the level in CSF on day 1 was 650.65 ng/ml, the plasma level on the same day was 202.50 ng/ml, and was within mean $\pm$ 2 SD obtained from 11 normal volunteers. The higher concentration in CSF was not simply explained by the elevation of the systemic plasma level. Our clinical and experimental findings therefore indicate the local, intracranial production of

this cholic acid. The possibility of extrahepatic production of cholic acid was previously reported in fibroblast by Skrede *et al.*¹¹⁾

A high concentration of 7 α -hydroxy-3-oxo-4-cholestenic acid was observed in both chronic subdural hematoma and CSF after aneurysmal SAH. Several clinical observations using computed tomography have shown that chronic subdural hematoma often arises at a pre-existing subdural effusion.^{5,6)} This fact suggests that the mixture of blood and CSF may occur in the chronic subdural hematoma at the initial stage. Therefore, the common factor in SAH and chronic subdural hematoma is the existence of a mixture of blood and CSF in the cranial cavity.^{5,6,13)} The high concentration of this compound found specifically in intracranial hemorrhagic disorders suggests a possible role for this compound in their pathogenesis. Further investigations are required to evaluate the possible biological activities of 7 α -hydroxy-3-oxo-4-cholestenic acid.

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