Cerebrospinal Fluid 20-HETE Is Associated With Delayed Cerebral Ischemia and Poor Outcomes After Aneurysmal Subarachnoid Hemorrhage

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Background and Purpose—Delayed cerebral ischemia (DCI) is a major complication after aneurysmal subarachnoid hemorrhage (aSAH); it is manifested by changes in cerebral blood flow accompanied by neurological decline, and it results in long-term functional and neuropsychological impairment. Preclinical evidence has demonstrated that the arachidonic acid metabolite, 20-hydroxyeicosatetraenoic acid (20-HETE), affects cerebral microvascular tone and cerebral blood flow after aSAH. The purpose of this study was to determine whether cerebrospinal fluid 20-HETE levels were associated with DCI and long-term neuropsychological outcomes in aSAH patients.

Methods—Cerebrospinal fluid samples were collected twice daily through 14 days after hemorrhage on 108 acute, adult, aSAH patients. Samples were analyzed for 20-HETE via HPLC MSQ single quadrupole mass spectrometry. DCI was defined as the presence of impaired cerebral blood flow (angiographic vasospasm, elevated transcranial Dopplers, abnormal computed tomography or magnetic resonance perfusion scans) accompanied by neurological deterioration. Outcomes, including death and neuropsychological testing, were completed at 3 months after hemorrhage.

Results—Detectable 20-HETE levels were observed in 31% of patient samples and were associated with severity of hemorrhage (Hunt & Hess [HH], \(P<0.04\); Fisher, \(P<0.05\)). Detection of 20-HETE was not associated with angiographic vasospasm (\(P=0.34\)); however, detectable 20-HETE was significantly associated with DCI (\(P=0.016\)). Our data also suggest that detectable 20-HETE was associated with decreased performance in 5 neuropsychological domains.

Conclusions—These results provide the first clinical evidence that cerebrospinal fluid 20-HETE concentrations are associated with DCI and poor outcomes, and this provides impetus for future studies to elucidate the clinical utility of inhibiting 20-HETE formation as a novel therapeutic intervention in patients with aSAH. (Stroke. 2011;42:1872-1877.)

Key Words: subarachnoid hemorrhage ■ delayed cerebral ischemia ■ 20-HETE ■ fatty acid ■ arachidonic acid

Aneurysmal subarachnoid hemorrhage (aSAH) is a devastating illness that strikes healthy individuals during active and productive years of their life and results in significant death and disability. Delayed cerebral ischemia (DCI) has been well accepted as a leading complication of aSAH that contributes to the overall morbidity and mortality, and is known to occur in approximately 20% to 60% of patients who survive initial hemorrhage.1–4 Although the definition of DCI continues to be refined, the development of DCI after aSAH is thought to result from a mismatch between available cerebral blood flow (CBF) and the metabolic needs of the brain tissue therefore, DCI can be determined by changes in CBF accompanied by neurological decline.5–8 This ischemic complication results in exacerbated long-term functional and neuropsychological (NP) impairment that interferes with resumption of previously held familial, social, and employment roles.8

Historically, cerebral vasospasm was considered the primary cause of DCI. Approximately 50% to 70% of aSAH patients have angiographic evidence of cerebral vascular constriction (angiographic vasospasm), and one third of these experience symptoms of DCI.1,8 However, not all patients that develop neurological decline with perfusion deficits have angiographic evidence of cerebral vasospasm.4,8–13 This clinical reality suggests that other causes of DCI in aSAH patients warrant exploration. DCI is most likely to occur 3 to 15 days after the initial bleeding, thereby allowing sufficient timing for therapeutic intervention. Unfortunately, to date, no ther-
apeutic intervention has been developed that significantly reduces the incidence of DCI.4

The exact mechanisms of DCI have not been fully elucidated, although recent evidence has linked metabolic regulators of cerebral microvascular blood flow as important mediators in its development.5,7,12 To date, therapies such as endothelin receptor antagonists have been effective in reducing angiographic vasospasm; however, their effect on DCI has not been as significant.3,11 Standardized care, including the use of calcium channel antagonists and triple H therapy (hypertension, hypervolemia, and hemodilution), may have contributed to the lower incidence or impact of DCI, but insight into risk or early identification of susceptible patients remains an important area of investigation.3,9,12

Recent evidence has suggested that a metabolite of arachidonic acid, known as 20-hydroxyeicosatetraenoic acid (20-HETE), may be influential in reduced CBF. 20-HETE is formed by enzymes of CYP4A and 4F families via ω-hydroxylation of arachidonic acid in cerebral arteries.14 Stimulated by angiotensin II, endothelin, and norepinephrine, 20-HETE is a potent microvascular vasoconstrictor in renal, mesenteric, and cerebral vascular beds. Although the majority of evidence is preclinical, 20-HETE has been implicated in changes in the cerebral vascular tone, including the development of delayed cerebral vasospasm and ischemia.15 Administration of inhibitors of 20-HETE synthesis and 20-HETE antagonists have been shown to reverse delayed vasospasm and to prevent acute decreases in CBF.14–15 Likewise, inhibition of 20-HETE formation was found to be neuroprotective in a temporary focal ischemia SAH animal model,20 and it attenuated the postinjury decrease in CBF that typically accompanies ischemic and hemorrhagic stroke.21

More recently, elevated levels of 20-HETE in cerebrospinal fluid (CSF) and plasma have been reported in a small number of aSAH patients with documented evidence of cerebral vasospasm and neurological deficits.16,22,23 Based on the preclinical evidence implicating 20-HETE in the development of both cerebral vasospasm and cerebral ischemia, the purpose of this study was to determine the time course of 20-HETE concentrations in the CSF of patients with aSAH and to determine whether 20-HETE CSF levels were associated with DCI and/or NP outcomes in a larger cohort of adult patients with aSAH.

Methods

Patient Sample
Adult patients age 18 to 75 years, diagnosed with aSAH via cerebral angiogram or head computed tomography were recruited from the neurovascular intensive care unit. Criteria for enrollment also included Fisher grade >1 and CSF access (ventriculostomy or lumbar drain). Patients were not enrolled if they had a history of debilitating neurological disease or SAH from trauma, mycotic aneurysm, or arteriovenous malformation. The protocol was approved by the Institutional Review Board, and informed consent was obtained from the patient or proxy before data collection.

Data Collection
Sociodemographics, including sex, age, race, severity of injury (Fisher Grade and HH Score), as well as clinical data, were collected from the medical record. CSF was withdrawn by registered nurses directly from the tubing each morning (8 a.m.±1 hour) and evening

Table 1. Tests of Neuropsychological Function by Domain

<table>
<thead>
<tr>
<th>Domain of NP Function</th>
<th>Test Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attention</td>
<td>Trail Making Test A</td>
</tr>
<tr>
<td>Verbal learning and memory</td>
<td>Digit Span Forward and Backward</td>
</tr>
<tr>
<td>Cognitive flexibility</td>
<td>Trail Making Test B</td>
</tr>
<tr>
<td>Visuospatial ability</td>
<td>Grooved Pegboard (Dominant and Non-dominant)</td>
</tr>
<tr>
<td>Language</td>
<td>Controlled Oral Word Association Test</td>
</tr>
</tbody>
</table>

NP indicates neuropsychological.

(7 p.m.±1 hour) through 14 days after initial hemorrhage (contingent on CSF access). Specimens were frozen and stored at −80 degrees for batch analysis. NP data were obtained through 1.5- to 2-hour face-to-face interviews conducted at 3 months after initial hemorrhage by an experienced clinician trained in NP assessment.

20-HETE Measurement

CSF aliquots of 1 mL were extracted via solid phase extraction using Oasis HLB 1cc extraction cartridges (Waters). 20-HETE was separated using HPLC with a 5 mm Beta Basic-C18 (150×2.1; ThermoHypersil) column and quantified using a MSQ single quadrupole mass spectrometer (ThermoFinnigan), under negative electrospray and single ion mode detection at mass m/z 319.5 for 20-HETE and m/z 325.5 for deuterated (d6)-20-HETE. Data were acquired and analyzed using Xcalibur software (version 1.0.0.1). Concentrations of 20-HETE were quantified from the standard curve of the ratio of 20-HETE to internal standard peak areas as previously described.23,24

DCI

DCI was defined as the presence of impaired CBF accompanied by neurological deterioration (simultaneously or within 12 hours pre- or postdetermination of impaired CBF). Neurological deterioration was determined by the presence of any of the following: a change in level of consciousness, presence of a new focal neurological deficit, pupil changes, or worsening Glasgow Coma Scale or National Institutes of Health Stroke Scale scores, documented by the bedside practitioner in the absence of medication administration. CBF was assessed by cerebral angiography, transtemporal Doppler, and computed tomography or magnetic resonance perfusion scans. Angiographic vasospasm was determined from cerebral angiograms read and coded by neurosurgeons blinded to participant identity and dichotomized as either “negative” (0%–24% narrowing of cerebral blood vessels) or “positive” (≥25% narrowing of cerebral blood vessels). Daily transtemporal Dopplers were coded as abnormal flows when there was a systolic middle cerebral artery velocity >200 mL/s and/or a Lindegaard ratio >3.0.1,2 Finally, head computed tomography/magnetic resonance and head computed tomography/magnetic resonance perfusion scans were reviewed for the presence of ischemia, infarction, or low blood flow. All patients received standard therapy for the study institution for SAH patients, including strict blood pressure and central venous pressure parameters, nimodipine, and triple H therapy.

NP Function

Seven domains of NP function were assessed (Table 1). The selected tests have excellent psychometric properties and have been used in large clinical trials studying the effects of multiple disorders on NP outcomes.26–27 To control for comorbidities, level of education was obtained from the participant or proxy, and premorbid intelligence was estimated using the North American Adult Reading Test; this
Data Analysis
Measurements of 20-HETE in cerebrospinal fluid were dichotomized into groups of detectable and nondetectable 20-HETE concentrations at baseline. Fisher exact tests and Mann-Whitney U tests were used to analyze the association between detectable and nondetectable levels of 20-HETE with sociodemographic, clinical, angiographic vasospasm, and DCI factors. A \( P < 0.05 \) was considered significant for assessment of 20-HETE relationships with sociodemographic and clinical factors. To analyze the effect of detectable and nondetectable 20-HETE levels on NP outcome scores, multivariate regression models were created using backwards linear regression, adjusting for age, sex, years of education, race, HH, and depression at baseline. For all NP assessments, a Bonferroni correction was applied, which meant that to be considered statistically significant, a relationship had to have a \( P < 0.002 \). All statistical analyses were performed using SPSS version 16 (SPSS, Inc).

Results
Patient Characteristics
A total of 108 aSAH patients were included in this analysis. The mean age (54 years; SD, 11.2) and the predominance of female patients (73%; \( n = 55 \)) reflect the known population of aSAH patients. The higher numbers of Caucasians (85%); \( n = 64 \) are representative of the population of the study site (Table 2). Overall impact of initial insult as graded by HH\(^3–5\) as well as by Fisher\(^1–4\) was moderate to severe. Mean duration of ventriculostomy placement was 7±3.4 days, yielding 11.7±6.5 CSF samples obtained per patient. Of the patients with DCI (\( n = 54 \)), a combination of vasospasm, demonstrated by cerebral angiography and/or transcranial Doppler, along with neurological deterioration was present in all but 1 patient (Table 3). The presence of new cerebral ischemia or infarct along with neurological decline was used in the other patient with DCI. DCI could not be determined on 9 patients (8%) because of poor neurological condition that excluded the ability to derive a neurological deterioration assessment.

Cerebrospinal Fluid 20-HETE Levels Following aSAH
20-HETE was detected in 33 aSAH patients (31%; Figure). There was no significant association between demographic variables (age, race, sex) and detectable 20-HETE concentrations (Table 2). There was a difference in detectable levels of 20-HETE concentration based on severity of injury graded by both HH score and Fisher grade. Approximately 85% of patients (\( n = 28 \)) with detectable levels of 20-HETE concentration had high HH scores, whereas 64% of patients (\( n = 48 \)) with nondetectable levels of 20-HETE concentrations had high HH scores (\( P = 0.04 \)). Similarly, only 9% of patients (\( n = 3 \)) with detectable levels of 20-HETE concentration had a Fisher Grade 2 compared with 29% of patients (\( n = 22 \)) with nondetectable levels (\( P = 0.05 \); Table 2).

CSF 20-HETE Levels and DCI
In this analysis, the presence of angiographic cerebral vasospasm alone was not associated with detectable levels of CSF 20-HETE (\( P = 0.34 \); Table 4). However, there was a significant relationship between the development of DCI and the presence of detectable CSF 20-HETE (\( P = 0.016 \)). In those with detectable CSF 20-HETE, 73% of patients (\( n = 22 \)) developed DCI, whereas only 46% of patients (\( n = 32 \)) with nondetectable levels of 20-HETE had DCI.

CSF 20-HETE Levels and NP Outcomes
The effect of CSF 20-HETE levels on NP outcomes at 3 months postinjury was assessed with multivariate linear regression models using a backwards selection procedure. Results indicated a trend towards poorer function in multiple NP domains for patients with detectable 20-HETE concentrations after controlling for age, sex, years of education, race, injury (HH), and depression. These domains included visuospatial ability (\( P = 0.03 \)), learning and memory (\( P < 0.01 \); \( P = 0.03 \)), language (\( P = 0.02 \)), attention (\( P = 0.02 \)), and mental flexibility (\( P < 0.01 \); Table 5). Because of the limited power of these preliminary findings, statistical significance was not
maintained after Bonferroni adjustment. As previously described, there was no association between mortality at 3 months and detectable 20-HETE levels (Table 2).

**Discussion**

This study demonstrates that 20-HETE CSF levels are associated with severity of injury and DCI in patients after aSAH. These results provide the first clinical evidence in a large cohort of patients that 20-HETE may be a pathogenic mediator of aSAH in humans. Previous smaller clinical studies by our laboratory and others have detected 20-HETE in CSF of aSAH patients and suggested that 20-HETE CSF levels are elevated in aSAH patients as compared with cerebrospinal fluid from healthy subjects. Furthermore, multiple preclinical studies have demonstrated that 20-HETE is a mediator of reduced cerebral blood flow after aSAH in both rat and dog models. Our current findings build on this previous work by demonstrating that 20-HETE is also associated with complications and outcomes in adult patients with aSAH.

Growing evidence has implicated 20-HETE in the pathogenesis of cardiovascular and neurovascular disease. 20-HETE has been shown to alter vascular smooth muscle tone upon activation by cytochrome P450 enzymes in the brain. The formation of 20-HETE is mediated by the CYP4F and CYP4A isoforms of the cytochrome P450 enzyme superfamily. In humans, CYP4F2 has demonstrated the greatest catalytic activity for 20-HETE formation. It has been suggested that 20-HETE is formed from arachidonic acid released during ischemia and cellular stress as opposing regulators of CBF and neuronal damage. In addition, certain single nucleotide polymorphisms that alter 20-HETE production have been associated with cerebral ischemia and hypertension in humans. The potential association of these variants with hemorrhagic stroke and/or 20-HETE CSF levels is an important area for future study.

Animal studies by our laboratory and others have demonstrated that inhibition of 20-HETE formation is neuroprotective in temporary focal ischemia and SAH models. These studies have also shown that 20-HETE inhibitors attenuate postinjury reductions in CBF that accompany both ischemic and hemorrhagic stroke. The mechanism of this neuroprotection has not been fully elucidated; however, it has been speculated that the protection is afforded by a decrease in 20-HETE production. 20-HETE has been shown to constrict blood vessels in the microvascular space, regulate new blood vessel growth, and augment vascular remodeling. Although 20-HETE has associated with microvascular constriction, it also dilates larger cerebral blood vessels. This is of significant interest in the current study because the presence of 20-HETE was associated with the development of DCI, but not with angiographic vasospasm. Several authors have suggested that cerebral angiography is more representative of macrovascular lumen changes and may not adequately explain the ischemic changes after aSAH. This limitation may explain the lack of significance between the presence of 20-HETE and angiographic evidence of vasospasm. Recent research related to outcomes after aSAH have moved away from cerebral vasospasm alone and have focused on measures of abnormal perfusion to explain DCI,

**Table 3. Determination of Delayed Cerebral Ischemia (DCI)**

<table>
<thead>
<tr>
<th>Delayed Cerebral Ischemia</th>
<th>20-HETE Group</th>
<th>Non-Detectable</th>
<th>Detectable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>N %</td>
<td>75 100</td>
<td>33 100</td>
</tr>
<tr>
<td>None</td>
<td>N %</td>
<td>37 49</td>
<td>8 24</td>
</tr>
<tr>
<td>Cerebral angiography and neurological</td>
<td>N %</td>
<td>1 1</td>
<td>2 6</td>
</tr>
<tr>
<td>deterioration</td>
<td></td>
<td>15 20</td>
<td>8 24</td>
</tr>
<tr>
<td>TCD and neurological deterioration</td>
<td>N %</td>
<td>16 21</td>
<td>11 33</td>
</tr>
<tr>
<td>Cannot identify: missing variable or</td>
<td>N %</td>
<td>6 8</td>
<td>3 9</td>
</tr>
<tr>
<td>poor exam</td>
<td></td>
<td>0 0</td>
<td>1 1</td>
</tr>
</tbody>
</table>

TCD indicates transcranial Doppler ultrasonography; MRI, magnetic resonance imaging; 20-HETE, 20-hydroxyeicosatetraenoic acid.

**Table 4. Association of Detectable and Non-Detectable 20-HETE Levels and Angiographic Vasospasm and Delayed Cerebral Ischemia**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>20-HETE Group</th>
<th>Non-Detectable</th>
<th>Detectable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiographic Vasospasm</td>
<td>N %</td>
<td>0.34</td>
<td>P</td>
</tr>
<tr>
<td>Total</td>
<td>48 100</td>
<td>25 100</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>27 56</td>
<td>11 44</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21 44</td>
<td>14 56</td>
<td></td>
</tr>
<tr>
<td>Delayed Cerebral Ischemia</td>
<td>N %</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>69 100</td>
<td>30 100</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>37 54</td>
<td>8 27</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>32 46</td>
<td>22 73</td>
<td></td>
</tr>
</tbody>
</table>

20-HETE indicates 20-hydroxyeicosatetraenoic acid.

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**Figure.** Overall profile of detectable 20-hydroxyeicosatetraenoic acid (20-HETE) concentrations in cerebrospinal fluid vs time during 14-day inpatient stays of aneurysmal subarachnoid hemorrhage (aSAH) patients (n=33).
including microvascular changes. In addition, there is a focused effort to uniformly define the clinical assessment methods of cerebral ischemia after aSAH. The findings of the current study provide the first evidence that 20-HETE may adversely impact symptomatic ischemic events in aSAH patients, possibly as a result of cerebral microvascular constriction.

Our study also provides preliminary evidence concerning the relationship between 20-HETE CSF concentrations and long-term outcomes after aSAH. Our observation that detectable 20-HETE CSF levels are associated with a trend towards poorer performance on NP outcomes in 5 domains (visuospatial, learning and memory, language, attention, and mental flexibility) was anticipated based on the proposed role of 20-HETE as a pathogenic mediator of aSAH. However, it is important to note that this study was powered for the evaluation of DCI and 20-HETE and therefore, evaluation of NP outcomes results should be considered preliminary; and as such, the significant relationships with outcomes did not withstand Bonferroni corrections. However, these preliminary results demonstrate the importance of additional exploration of these relationships with a larger cohort of patients.

**Limitations**

One limitation of this study is that our evaluated population requires access to CSF. Patients who require placement of external ventricular catheters represent a subgroup of aSAH patients who may have higher risk of complications after aSAH because of the extent of injury, including DCI. A second limitation is caused by the lack of consensus as to the definition of DCI. In this analysis, we defined DCI via components of altered blood flow in the presence of neurological deterioration. Although the definitions are similar, more definitive criteria for DCI have been published since this analysis, which will be vital in future studies. Not all patients in this analysis had angiographic data available, as it is performed as a result of clinical changes rather than for research purposes; therefore, the correlation with angiographic vasospasm is with a subset of this patient sample. Furthermore, determining a clinical change on a patient with poor neurological condition from the onset of injury limits the use of neurological assessment used to determine whether the patient has symptomatic ischemia. Of note, development of neurological symptoms greater than 1 hour that has been suggested in more recent literature was not considered in this analysis, but may be an important consideration in future studies. Finally, the level of 20-HETE that we observed required statistical analysis of detectable versus nondetectable concentrations to assess the relationship with DCI and NP outcomes. Future studies will focus on the temporal profile of 20-HETE to better elucidate the timing of elevated 20-HETE concentrations relative to the onset of DCI.
Conclusions

In this study, we observed that the presence of CSF 20-HETE was associated with DCI and poor neurological outcomes in aSAH patients. Based on this finding, we conclude that 20-HETE may be involved in the pathogenesis of aSAH in humans. These results support the need for continued investigation of the role of 20-HETE and aSAH. Specifically, future studies employing more sensitive analytical methods will allow for improved sensitivity and evaluation of the temporal profile of 20-HETE concentrations in patients after aSAH.

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Disclosures

None.

References

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