Exploring the Age of Intracranial Aneurysms Using Carbon Birth Dating

Preliminary Results

Nima Etminan, MD; Rita Dreier, PhD; Bruce A. Buchholz, PhD; Peter Bruckner, PhD; Hans-Jakob Steiger, MD; Daniel Hänggi, MD; R. Loch Macdonald, MD, PhD

Background and Purpose—There is a controversy about the time span over which cerebral aneurysms develop. In particular, it is unknown whether collagen in ruptured aneurysms undergoes more rapid turnover than in unruptured aneurysms.14C birth dating of collagen could be used to address this question.

Methods—Aneurysmal domes from patients undergoing surgical treatment for ruptured or unruptured aneurysms were excised. Aneurysmal collagen was isolated and purified after pepsin digestion. Collagen from mouse tendons served as controls. F14C levels in collagen were analyzed by accelerator mass spectrometry and correlated with patient age and aneurysm size.

Results—Analysis of 10 aneurysms from 9 patients (6 ruptured, 3 unruptured) revealed an average aneurysm collagen age of <5 years, generally irrespective of patient age and aneurysm size or rupture status. Interestingly, F14C levels correlated with patient age as well as aneurysm size in ruptured aneurysm collagen samples.

Conclusions—Our preliminary data suggest that collagen extracted from intracranial aneurysms generally has a high turnover, associated with aneurysm size and patient age. The correlation of patient age and aneurysm F14C levels could explain models of aneurysm development. Although preliminary, our findings may have implications for the biological and structural stability of ruptured and unruptured intracranial aneurysms. (Stroke. 2013;44:799-802.)

Key Words: age ■ intracranial aneurysms ■ radiocarbon birth dating

Subarachnoid hemorrhage attributed to ruptured intracranial aneurysms has a poor prognosis, with most patients suffering permanent morbidity and case fatality remaining between 25% and 50%.1 The poor outcomes associated with aneurysm rupture support the treatment of unruptured aneurysms, but this is complicated by the low risk of rupture of small unruptured aneurysms. Although ruptured aneurysms are also frequently small, the low risk of rupture has stimulated an interest in the formation and progression of aneurysms and has sparked controversy about the time span over which aneurysms or their main component, collagen, might develop.14C birth dating enables an estimate of the age of aneurysm collagen. Natural 14C production was essentially constant over 2000 years until atmospheric testing of nuclear weapons from 1950 to 1963 produced a global 14CO2 bomb pulse. After the 1963 nuclear test ban, atmospheric 14C levels decreased exponentially because of migration of 14C into the biosphere. Consumption of plants and animals results in 14C levels in the human body parallel to those in the atmosphere with regional 14C levels almost homogeneous since late 1960s. Hence, 14C concentration can date any biomolecule using accelerator mass spectrometry (Figure 1A). Retrospective birth dating of neurons and fat cells has been reported.2,3 In this pilot study, we investigate the feasibility of birth dating of aneurysm collagen from patients undergoing surgical treatment for unruptured or ruptured intracranial aneurysms.

Methods
This study was approved by the research ethics committees of the Medical Faculty of Heinrich-Heine University, Düsseldorf, Germany (ID No. 3365); St. Michael’s Hospital, Toronto, Canada (ID No. 09–309); and Lawrence Livermore National Laboratory, Livermore, California (No. 10–108). After informed consent, samples from aneurysm domes (>3 mm) from 9 consecutive patients undergoing surgical treatment for a ruptured or unruptured aneurysm were surgically excised when feasible and kept frozen at –80°C until collagen extraction. Collagen samples with known age from new born mouse tendons and skin served as controls.

Collagen Purification
Collagen was isolated from aneurysm domes and purified using pepsin digestion. Tissue fragments were minced and suspended in at least 1 mL of 20 mmol/L HCl. Collagens were extracted by digestion with 100 μg pepsin/g of tissue at 4°C for 72 hours and were...
precipitated for 6 hours after addition of solid NaCl to a final concentration of 2.5 mol/L. After centrifugation, collagen was redissolved in 20 mmol/L HCl and exhaustively dialyzed against 20 mmol/L HCl. Collagen quantity and purity were assessed by SDS-PAGE.

Birth Dating of Aneurysm Collagen

All 14C accelerator mass spectrometry analyses were performed blinded to the age of individual patients and rupture status of the aneurysm. Purified collagen samples were transferred to quartz combustion tubes and lyophilized. Excess copper oxide was added to each dry sample, and tubes were evacuated and sealed with a H2/O2 torch. Samples were combusted at 900°C for 3.5 hours and evolved CO2 was purified, trapped, and reduced to graphite in the presence of iron catalyst. 14C in the graphite was measured at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory using standard techniques. The concentration of 14C/C was expressed using the F14C nomenclature±1 SD.4,5 The intercept date range corresponds to the 2 SD range of atmospheric 14C/C mapped onto the chronological record. The measurement error, determined for each sample, was accurate to 0.3% to 0.8%, corresponding to a chronological uncertainty of 1 to 3 years.4

Statistical Analysis

For ruptured aneurysms, the relation among patient age, aneurysm size, and F14C levels were investigated using Pearson product-moment correlation for metric or continuous variables. Significance was accepted at a level of P≤0.05. Statistical analysis was performed using SPSS 15.0.1 (Ulead Technologies, Chicago, IL). All values are means±SD.

Results

Between September 2009 and November 2011, 10 aneurysm domes (7 ruptured, 3 unruptured, mean aneurysm size 13±10 mm) were excised from 9 patients (mean age 63±12 years) who underwent surgical clipping. One aneurysm was treated by trapping, allowing the excision of 2 larger samples, one of which was taken from the neck of the aneurysm (R3 and R7). Pepsin digestion of aneurysm tissue yielded sufficient amounts of collagen (mean carbon yield =0.46±0.31 mg) for further birth dating in all samples. A representative electrophoretic pattern of pepsin-resistant aneurysm protein revealed highly enriched collagens I and V. (Figure 2). The 14C concentrations of collagen were placed on the 14C record with recent unpublished values and projected concentrations to 2017 to determine intercept age ranges.6-8 All collagen samples derived from the ruptured or unruptured aneurysms were <5 years of age, irrespective of aneurysm rupture status, aneurysm size, and patient age (Table I, Figure 1B and 1C). However, in patients with ruptured aneurysms, there was a correlation between F14C levels and patient age (R2=0.93, P<0.001), as well as aneurysm size (R2=0.86, P<0.05). The mouse collagen controls (skin F14C =1.0323±0.0025, knee tendon F14C =1.0366±0.0037, knee tendon F14C =1.0316±0.0037) were within 2 SDs of the mouse chow F14C =1.0310±0.0036, which validates the birth dating method.

Discussion

This pilot study reveals that aneurysm collagen was <5 years old, that is, distinctly younger than the patients harboring the aneurysms. In addition, there was no obvious difference in collagen age between ruptured and unruptured aneurysms. Interestingly, F14C levels correlated with patient age and aneurysm size in patients with ruptured aneurysms. Previous mathematical or hemodynamic models or short-term observational studies gave only limited information on

Figure 1. A. Atmospheric 14CO2 levels have been essentially stable over the past 2000 years, except for a large increase between 1955 and 1963 owing to atmospheric nuclear testing. The Δ14C nomenclature corrects for radioactive decay and shows the historical 14C production rate.4 B–C. The collagen samples of each individual patient were placed on the 14C record with projected concentrations to 2017 expressed in the F14C nomenclature.4,6-8 Paired birth dates (vertical lines) and collagen date ranges are depicted for ruptured (R) and unruptured (U) aneurysms.

Figure 2. Electrophoretic analysis of pepsin digested aneurysmal collagens. A representative Coomassie brilliant blue-stained SDS-page gel (4.5% to 15%, reducing conditions) is shown. The predominant bands represent α bands of collagen type I (α1[I], α2[I]) and type V (α1[V], α2[V]), as well as β components of collagen type I (β1[I], β1.2[I]).
Table 1. Aneurysm- and Patient-Characteristics and Intercepted Ages

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Aneurysm Location</th>
<th>Patient Age</th>
<th>Date of Surgery</th>
<th>Date of Rupture (SAH)</th>
<th>Aneurysm Diameter, mm</th>
<th>F14C</th>
<th>Intercept Year Range</th>
<th>Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>MCA</td>
<td>53</td>
<td>Jan 20, 2010</td>
<td>Jan 20, 2010</td>
<td>Yes</td>
<td>5</td>
<td>1.0348±0.0036</td>
<td>2010–2015</td>
</tr>
<tr>
<td>U1</td>
<td>MCA</td>
<td>74</td>
<td>Jan 21, 2010</td>
<td>n/a</td>
<td>No</td>
<td>13</td>
<td>1.0534±0.0039</td>
<td>2007–2009</td>
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<tr>
<td>R2</td>
<td>PICA</td>
<td>57</td>
<td>April 1, 2010</td>
<td>April 1, 2010</td>
<td>Yes</td>
<td>8</td>
<td>1.0431±0.0038</td>
<td>2008,3–2012</td>
</tr>
<tr>
<td>R3</td>
<td>PICA</td>
<td>77</td>
<td>Sep 1, 2009</td>
<td>Sep 01, 2009</td>
<td>Yes</td>
<td>12</td>
<td>1.0573±0.0039</td>
<td>2005,3–2008</td>
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<td>R7</td>
<td>PICA</td>
<td>77</td>
<td>Sep 1, 2009</td>
<td>Sep 01, 2009</td>
<td>Yes</td>
<td>12</td>
<td>1.0516±0.0038</td>
<td>2006,3–2010</td>
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<tr>
<td>U2</td>
<td>MCA</td>
<td>48</td>
<td>July 5, 2010</td>
<td>n/a</td>
<td>No</td>
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<td>1.0505±0.0068</td>
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<td>U3</td>
<td>PCA</td>
<td>74</td>
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<td>No</td>
<td>15</td>
<td>1.0361±0.0039</td>
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<tr>
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<td>53</td>
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<td>Jan 26, 2011</td>
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<td>8</td>
<td>1.0370±0.0039</td>
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</tr>
<tr>
<td>R6</td>
<td>ACA</td>
<td>51</td>
<td>Nov 6, 2011</td>
<td>Nov 06, 2011</td>
<td>Yes</td>
<td>7</td>
<td>1.0330±0.0044</td>
<td>2010,6–2015</td>
</tr>
</tbody>
</table>

ACA indicates anterior cerebral artery; MCA, middle cerebral artery; n/a, not available; PICA, posterior inferior cerebellar artery; and SAH, subarachnoid hemorrhage. F14C nomenclature is expressed as fraction modern carbon and compares 14C/C in a sample to that in the atmosphere before the industrial revolution (F14C=1). The intercept date range corresponds to the 2 SD range of atmospheric 14C/C mapped onto the chronological record of atmospheric 14C measurements.

the temporospatial development of aneurysms.9–11 Most studies suggest that ruptured intracranial aneurysms undergo nonlinear growth or progression before rupture, suggesting that ruptured aneurysms have progressed relatively recently. This is supported by the high number of small aneurysms found after subarachnoid hemorrhage in epidemiological studies.12 According to some of these models, the tissue should be older and more stable in unruptured aneurysms. Our study provides the first actual estimate for the actual biological age of intracranial aneurysms at the time of treatment.

Although the number of samples investigated here is limited, the results suggest that all intracranial aneurysms have a high collagen turnover, regardless of their rupture status. However, the data from the ruptured aneurysms also suggest that larger aneurysms are slightly older which indicates that our method is valid because larger aneurysms should take longer to form. We have assessed the age of collagens I and V which are the main molecular components of the aneurysm mass.13 An important question is whether the collagen turnover in cerebral aneurysms is different from that of their parent artery. In cerebral arteries, structural stability is maintained by contractile elements (ie, smooth muscle cells) whose turnover is low.14 By contrast, cerebral aneurysms are structurally less stable and, hence, are prone to rupturing.15 Additionally, the correlation of patient age and F14C levels underlines assumptions on development of ruptured aneurysms. Ruptured aneurysms of older patients seem to contain slightly older collagen, which implicates that if an aneurysm does not rupture at an early stage, it may stabilize, although it may either grow and/or rupture subsequently. Alternatively, collagen may turnover more slowly in older patients’ ruptured aneurysms per se, whereas collagen may undergo more rapid turnover, that is, formation progression in younger patients. Although this has experimentally been solely studied for cerebral arterial walls, data from observational aneurysm studies do not support this hypothesis.9,15

Some questions can be raised about this data. Birth dating of collagen derived from cerebral aneurysms can only be performed in patients undergoing surgical clipping. Even though collagen is a major component of aneurysms, there might be a difference between the age of aneurysm collagen and the aneurysm itself. Also, we do not know whether there are differences in the age of different parts of the aneurysm. The limited number of birth dated aneurysms does not permit firm conclusions at this point. Finally, 14C birth dating of aneurysms can only provide chronological data with an uncertainty of up to 3 years. The correlations shown between aneurysm and patient age, as well as aneurysm size, are of uncertain clinical importance, given the small sample size and the small differences in age of collagen (2–3 years). Whether increased temporal resolution could provide more insight into differences in the development of ruptured and unruptured aneurysms cannot be answered at present.

Conclusions

14C birth dating of aneurysm collagen is feasible. Our data suggest that collagen from ruptured or unruptured intracranial aneurysms is distinctively younger than the corresponding patients. Furthermore, F14C levels in ruptured aneurysms strongly correlated with aneurysm size and patient age, underlying previous mathematical models on chronological aneurysm development. Thus, F14C birth dating of aneurysms warrants more investigation to potentially understand the development and structural stability of ruptured and unruptured intracranial aneurysms.

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References
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