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LLNL-JRNL-641775

Cerebral Aneurysms: Formation, Progression and Developmental Chronology

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August 1, 2013

Translational Stroke Research

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Introduction

The prevalence of unruptured intracranial aneurysms (UAIs) is assumed to be up to 3% of the general population, with 20-30% of patients harboring more than one aneurysm. [1-3]. While there is no data suggesting an increase of the prevalence of UIAs over the past decades, it seems that UIAs are increasingly detected, probably due to the higher distribution and technological improvement of imaging devices (i.e. magnet resonance imaging and computerized tomography) and also increasing scans in patients due to unspecific symptoms, such as headaches or dizziness. [4-7] UIAs can remain clinically silent, become symptomatic due to local mass effect or seizures or progress towards rupture. The case fatality of aneurysmal subarachnoid hemorrhage due to a ruptured aneurysm remains 25-50%. The poor prognosis and moreover the existing uncertainty regarding the natural history of UIAs continues to motivate researchers to explore mechanisms of aneurysm formation and progression as well as to better understand chronological development of UIAs. Here, we review the existing knowledge in this respect.

Aneurysm formation

Under physiological conditions cerebral arteries consist of three layers (from inside to outside): A) the intima with the basal membrane and endothelial cells, B) the media, consisting of circumferentially oriented smooth muscles cells, embedded into a dense network of collagen and elastin fibers that enable compliance and C) the adventitia, which mainly consists of collagen, providing the structural integrity of the vessel wall. [8] Importantly, the intima and media are separated by the internal elastic lamina, which is believed to be the origin of aneurysm formation on the structural level. [8] Intracranial blood vessels are somewhat different, when compared to extracranial vessels, because of their thicker internal elastic lamina, decreased proportion of elastin fibers and smooth muscle cells in the media and the thinner adventitia. [9] This together with the lower amount of connective tissue within subarachnoid space itself makes cerebral arteries more prone to develop an aneurysm per

se. Current hypotheses on cerebral aneurysm formation postulate the following mechanisms. First, initial apoptosis in vascular smooth muscle cells within the vessel wall and disruption of the elastic internal lamina. Second, collagen fiber reconstitution due to the resulting shift in tensile forces leads to subsequent collagen and/or elastin degradation as well as their break-down leading to vessel wall remodeling. [10, 11] Whether this remodeling will then result in aneurysm sack stabilization, progression or even rupture is assumed to be dependent on the degree of aneurysm wall inflammation, hemodynamic stress due to cardiovascular risk factors (e.g. hypertension or nicotine consumption) and remodeling/turnover of tissue and collagen in the aneurysm wall. [10, 12-14] The relevance of the inflammatory component in the pathogenesis of aneurysm progression and rupture is becoming increasingly evident in more recent studies. [15-21] The hypothesis that the incidence and degree of an inflammatory reaction may determine aneurysm rupture more strongly, as opposed to aneurysm size or location, is supported by the fact that the induction and progression of cerebral aneurysms can be significantly reduced following administration of anti-inflammatory drugs *in vivo*. [22, 23] Additionally, there seems to be a protective effect of anti-inflammatory drugs with respect to cerebral aneurysm rupture in patients with UIAs. [24, 25]

Predisposing factors to develop a cerebral aneurysm

A genetic predisposition to develop a CA must be assumed due to increased familial occurrence and association with hereditary conditions (e.g. autosomal dominant polycystic kidney disease) but a specific candidate gene linked to development of cerebral aneurysms has not yet been identified. [26] Genome-wide linkage studies in cohort with familial aneurysms have led to identification of several loci on chromosomes, which were strongly linked to CAs in patients with familial CAs: 1p34.3-p36.13, 19q13.3, Xp22 and 7q11. [4, 27, 28] Future studies on positional candidate genes from these regions may then help to identify people at increased of developing a CAs, within the general population. [26] The gene with robust

evidence of linkage is located on 7q11, which is close to the elastin gene, and thus associated with structural integrity of the arterial wall. [29, 30] 7q11 also contains the gene for collagen type 1 A2, which also fundamentally contributes to the integrity of the vascular wall. [30] Nevertheless, the present data remains to be validated with larger sample sizes in ethnicity diverse populations until large geneticly-based screening studies are warranted. [30, 31]

Interestingly, about 20% of those patients harboring an UIA have a positive family history for a unruptured or ruptured CA. [32, 33] Conversely, the prevalence of UIAs in families with at least two members with cerebral aneurysms is as high as 19.1%, compared to 2-3% in the general population. [2, 34] Aside from patient age and duration of coexistent hypertension, the incidence of aneurysms in these patients is markedly associated the amount of nicotine consumption and gender: Individuals with current nicotine consumption within families with a positive history for intracranial aneurysms, had 3-fold risk of harboring an UIA, compared to patients without nicotine consumption. Further, the odds for a positive UIA screening were twice as high in females (OR 2.46). [34] This underlines that in addition to a familial predisposition, modifiable risk factors are strong determinants for whether or not an aneurysm is formed throughout time and that serial radiological screening in such patient cohorts may be warranted. Nevertheless, the complex pathophysiology of cerebral aneurysm formation remains incompletely understood, since our current knowledge in this respect is mainly derived from *in-vivo* - or mathematical- models or observational studies. [12, 14, 35-40]

Aneurysm growth and Progression intervals

De novo formation and growth or progression of cerebral aneurysm in serial imaging are important surrogates for instability of an UIA. [7, 41] Here, more knowledge is important to better understand the natural history of UIAs but somewhat difficult to obtain, as the majority of present data is derived from short-term follow-up studies, mostly in patients who already had a SAH from a different aneurysm; [14, 36, 37, 39, 40, 42-44] Additionally, this data is somewhat biased as a) patients with previous SAH are more prone to develop another aneurysm or even SAH,

are b) usually younger and c) more likely to have hypertension or nicotine as a risk factors, compared to the general population. [36] Irrespective of this potential bias, the currently assumed annual rate of the novo aneurysm formation ranges from 0.3-1.8% in these populations. [36, 39, 40, 42, 44] The most relevant risk factors for de novo aneurysm formation in theses cohorts were female gender, nicotine consumption, aneurysm multiplicity, patient age and longer follow-up duration. [43] The annual incidence of aneurysm growth in previous studies ranged from 1.51-22.7%. [43] In addition to aforementioned risk factors for aneurysm formation, an important risk factors for aneurysm growth is aneurysm size per se: Here, the cut-off sizes for increased risk of aneurysm growth ranged from 5 to 10mm. [43] For UAIs there is data suggesting rather inconstant, non-linear aneurysm growth: Using population-based SAH incidence rates, different mathematical scenarios were simulated to investigate aneurysm growth rate and it was found, that aneurysms are unlikely to grow at constant, time-independent rates. Further, periods of aneurysm growth seem to be much shorter and less frequent than periods without such growth, as only 1 in 4 persons was likely to display aneurysm growth over 6.7 years. [37, 40] Nevertheless, the rate of de novo aneurysm formation and aneurysm growth in the general population may or may not be distinctly higher as in SAH patient cohorts but the chronological development of aneurysms has been difficult to estimate because of the lack of data from serial imaging in such populations. [36] However, more recently, we reported the feasibility to analyze chronological development and/or turn-over in human aneurysmal tissue in a pilot series using radiocarbon birth dating. [45]

Accelerator mass spectrometry to measure chronological tissue turn-over

Accelerator mass spectrometry (AMS) is a technique for measuring part per trillion levels of rare long-lived radioisotopes such as ^{14}C. [46, 47] AMS measures traces of anthropogenic and naturally occurring ^{14}C in proteins to measure the time at which the protein was formed. ^{14}C is produced naturally by the interaction of cosmic radiation and ^{14}N in the atmosphere. The systematic radioactive decay of ^{14}C (radioactive half-life $T_{1/2}$=5730 y) is utilized in traditional

radiocarbon dating. Natural ^{14}C production has varied only slightly over the past 4000 years (FIGURE 1 A). [48] However, above ground nuclear testing produced a sharp and global increase of atmospheric ^{14}C levels between 1955 and 1963. [49-52]This excess is often referred to as the radiocarbon bomb pulse. Whether a result of natural or anthropogenic processes, newly produced ^{14}C in the atmosphere is rapidly oxidized to $^{14}CO_2$. It then subsequently enters the food chain as$^{14}CO_2$ and is incorporated into the biosphere. After the ban on above ground nuclear testing in 1963, the atmospheric ^{14}C levels have exponentially dropped, not because of radioactive decay but as a result of diffusion and equilibration of ^{14}C with the biosphere and oceans. The consumption of plants and animals that live off plants leads to ^{14}C levels in the human body parallel to those in the atmosphere. [53-55] ^{14}C can be measured to determine the age of any biomolecule using AMS, provided that the sample's purity is high. The levels of ^{14}C in proteins reflect the atmospheric ^{14}C levels at the time at which the protein was formed, which corresponds to the protein age that is then used to calculate turnover rate that protein. [56, 57] Importantly, AMS counts atoms and not radioactive decay, which is more efficient and faster. [46] The measurement precision is 0.2-0.8%, which corresponds to a chronological uncertainty of ± 1-3 years in recent years.[57] Cell birth dating has been performed by measuring the ^{14}C content of DNA for different human tissue types or cells, such as neurons, adipocytes, cardiomyocytes,, beta cells. [58-63] Proteins and lipids have been dated to assess turnover and growth of pathological structures. [56, 57, 64]

^{14}C birth dating of cerebral aneurysms

For more detailed methodology please refer to the actual publication. [45] Radiocarbon birth dating of cerebral aneurysms has the following prerequisites: A) sufficient amount of aneurysm tissue, B) a specific molecule representative of aneurysm age, and C) a specific biomolecule or class of molecules that can be separated to high purity with robust yield. Based on these prerequisites, collagen is the best molecule for aneurysm dating since it constitutes the main component of the aneurysmal mass and can be isolated and purified using a modified pepsin digestion assay. Furthermore, collagen is routinely harvested from

bone for traditional radiocarbon dating, so techniques to produce high purity collagen free of carbon contamination suitable for AMS analyses are known. [34] Following surgical repair of ruptured or unruptured aneurysms, we excised aneurysm domes and froze them at -80°C. The aneurysms were on average larger than 4-5mm due to patients safety and to ensure sufficient amounts of collagen. For the ^{14}C birth dating of the aneurysm tissue using AMS, we isolated and purified collagen types I and V from the aneurysmal sack with pepsin based on the modified Longin method. [65] In a first step, we analyzed the samples for content and purity using SDS-PAGE electrophoresis and verified that the main proteins after pepsin digestion and purification of the aneurysms were collagen types I and V (not shown). In a second step, collagen samples were lyophillized and tendons from new born mice served as process controls to ensure collagen isolation did not add significant carbon contamination due to the method.

AMS sample preparation and measurement procedures vary slightly among AMS facilities but all employ the same general process of combustion to CO_2 followed by reduction to elemental carbon (graphite) when measuring solid samples. Dried collagen samples are transfered to quartz combustion tubes with excess copper oxide, the tubes evacuated and sealed using a H_2/O_2 torch Sealed quartz tubes are placed in an oven at 900°C for 3.5 h to combust carbon to CO_2. The CO_2 is cryogenically purified to remove water and reduced to elemental carbon on Fe or Co metal powder catalyst with H_2. Graphite samples and sputtered with Cs^+ ions and negative ions are extracted at generally 40-60 keV. Samples, isotopic standards, and controls are measured to better than 1% precision.

In a pilot study 7 ruptured and 3 unruptured aneurysms were retrieved from 9 patients following surgical clipping. The yield of collagen from the aneurysm tissue was sufficient for further analysis (mean 0.46 ± 0.31 mg) in all samples. As illustrated in figure 1, the ^{14}C concentrations of collagen were placed on the ^{14}C record with the projected concentrations to 2017 to determine intercept age ranges. The preliminary data suggested that aneurysm collagen was distinctly younger than the individual patient, which harbored the aneurysm,

since we did not find an aneurysm sample, which was older than 5 years. If verified in a larger patient cohort, these findings suggest, that there is constant collagen turn-over in cerebral aneurysms rather than that aneurysm development shorty before their clinical presentation. However, due to the small sample size, we have no knowledge whether factors such as patient age, aneurysm size and location or rupture status might determine the turnover of aneurysm. Additionally, the current method holds several limitations, as the measurement precision corresponds to chronological uncertainty up to 3 years and since we cannot estimate whether sample closer to the aneurysm neck, i.e. where aneurysms form, may actually be older. Further, we do not have any knowledge on the relationship of collagen turn-over in aneurysm and their parent arteries, since parent artery cannot be removed in the surgeries that removed the aneurysms .

Nevertheless, our preliminary data suggest that aneurysm undergo constant remodeling, which may challenge hypotheses that cerebral aneurysms are generally stable and old entities. At present, it remains to be elucidated which epidemiological or morphological factors determine aneurysm turnover.

CONCLUSION

The pathogenesis of cerebral aneurysm formation is a multifactorial and incompletely understood process. At present, it remains unknown what the actual cause for the intial disruption of the elastic internal lamina in the arterial vessel wall is and whether or not this process occurs early or later throughout the patients lifetime. However, the current knowledge on chronological aneurysm development suggests that the progression of aneurysms and remodeling of aneurysm tissue is a discontinuous but ongoing process and that cerebral aneurysm cannot be generally assumed to be stable lesions. To understand the

true natural history of cerebral aneurysms, future studies should investigate determinants for their chronological formation and growth.

Figure legends:

Figure 1 A) Atmospheric $^{14}CO_2$ levels have been essentially stable over the past 2000 years, except for a large increase between 1955-1963 due to above ground nuclear tests.B, C) The collagen samples of each individual patient were placed on the ^{14}C record with projected concentrations to 2017 expressed in the $F^{14}C$ nomenclature, which corrects for radioactive decay and illustrates above ground ^{14}C levels over the past 2000 years . [48-50, 66] The patients' birth dates (vertical lines) and the aneurysm collagen date ranges (horizontal lines) are illustrated for ruptured (R) and unruptured (U) samples.

Figure 2 The average age of cells can be determined from the $^{14}C/C$ content of the DNA of the cell population. The age of tissues (red) and specific cell types (orange) are depicted for a human subject who was born in 1973 (vertical line) and died in 2005 based on published turnover rates..[59, 61-63, 67, 68]

FIGURES

Figure 1

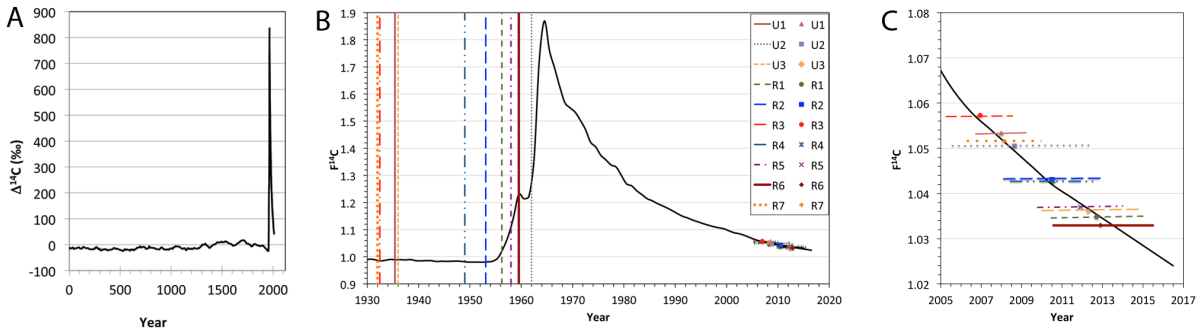
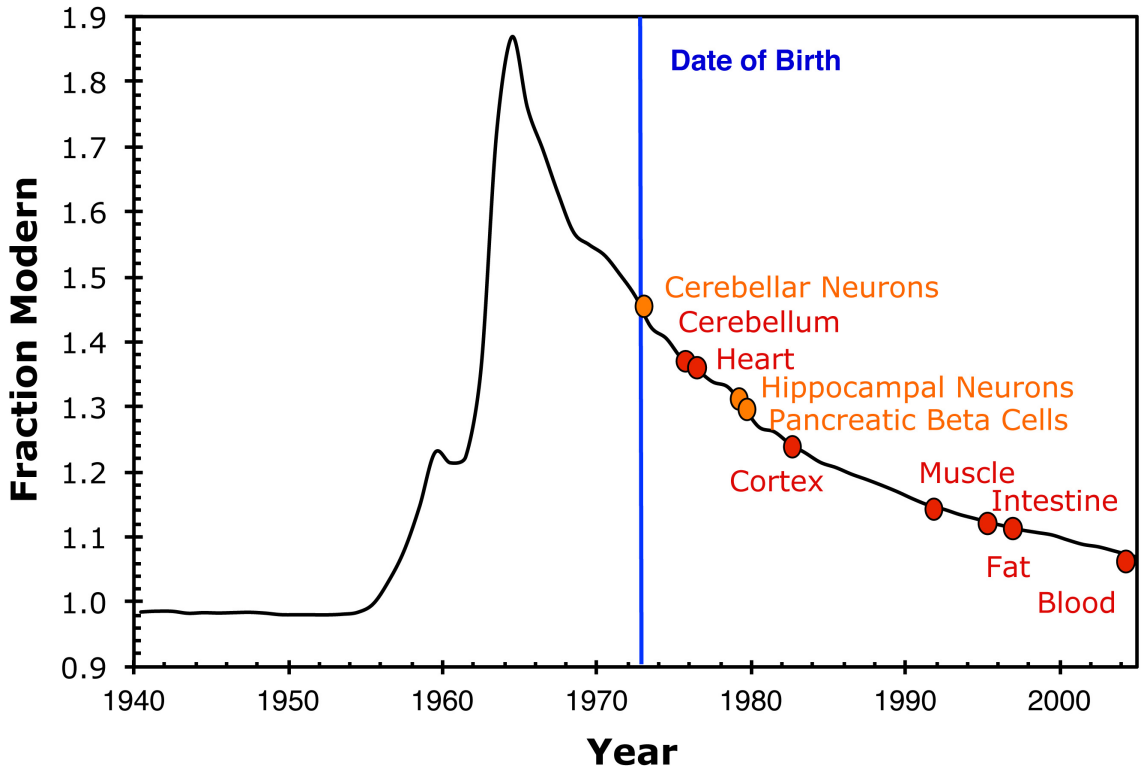


Figure 2



DISCLOSURES and ACKNOWLEDGMENTS

NE and RLM receive grant support from the Physicians Services Incorporated Foundation. RLM receives grant support from the Brain Aneurysm Foundation, Canadian Institutes of Health Research and the Heart and Stroke Foundation of Ontario. RLM is a consultant for Actelion Pharmaceuticals and Chief Scientific Officer of Edge Therapeutics, Inc.
NE, DH and RLM are scientific advisors/officers for Edge Therapeutics, Inc.

Support was also provided by NIH/NIGMS 8P41GM103483. This work was performed in part under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344

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