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Aging is associated with changes to the biomechanical properties of the posterior cerebral artery and parenchymal arterioles

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Diaz-Otero JM, Garver H, Fink GD, Jackson WF, Dorrance AM. Aging is associated with changes to the biomechanical properties of the posterior cerebral artery and parenchymal arterioles. Am J Physiol Heart Circ Physiol 310: H365–H375, 2016. First published December 4, 2015; doi:10.1152/ajpheart.00562.2015.—Artery remodeling, described as a change in artery structure, may be responsible for the increased risk of cardiovascular disease with aging. Although the risk for stroke is known to increase with age, relatively young animals have been used in most stroke studies. Therefore, more information is needed on how aging alters the biomechanical properties of cerebral arteries. Posterior cerebral arteries (PCAs) and parenchymal arterioles (PAs) are important in controlling brain perfusion. We hypothesized that aged (22–24 mo old) C57Bl/6 mice would have stiffer PCAs and PAs than young (3–5 mo old) mice. The biomechanical properties of the PCAs and PAs were assessed by pressure myography. Data are presented as means ± SE of young vs. old. In the PCA, older mice had increased outer (155.6 ± 3.2 vs. 169.9 ± 3.2 μm) and lumen (116.4 ± 3.6 vs. 137.1 ± 4.7 μm) diameters. Wall stress (375.6 ± 35.4 vs. 504.7 ± 60.0 dyn/cm²) and artery stiffness (β-coefficient: 5.2 ± 0.3 vs. 7.6 ± 0.9) were also increased. However, wall strain (0.8 ± 0.1 vs. 0.6 ± 0.1) was reduced with age. In the PAs from old mice, wall thickness (3.9 ± 0.3 vs. 5.1 ± 0.2 μm) and area (591.1 ± 95.4 vs. 852.8 ± 100 μm²) were increased while stress (758.1 ± 100.0 vs. 587.2 ± 35.1 dyn/cm²) was reduced. Aging also increased mean arterial and pulse pressures. We conclude that age-associated remodeling occurs in large cerebral arteries and arterioles and may increase the risk of cerebrovascular disease.

aging; cerebrovascular circulation; remodeling; vasculature

NEW & NOTEWORTHY

Aging is associated with changes to the biomechanical properties of parenchymal arterioles and posterior cerebral arteries; this could compromise cerebrovascular health and increase the risk of stroke and dementia. Our studies are novel because of the advanced age of the mice studied and the analysis of the parenchymal arterioles.

AGING is characterized by a decline in many physiological and vascular functions (5). Artery dysfunction (23) is an important factor in cardiovascular diseases such as hypertension, atherosclerosis, and cerebral artery disease, which are major causes of mortality in the elderly (36). The incidence of cardiovascular and cerebrovascular disease increases significantly with age; this is especially true for stroke and heart failure (24, 25). The remodeling of arteries that occurs with age may contribute to this association between age and cardiovascular disease (31). The term “artery remodeling” refers to stable changes in artery diameter and wall structure; inward remodeling is a reduction in lumen diameter while outward remodeling refers to an increase in lumen diameter. Hypertrophic remodeling occurs when wall area is increased, while hypotrophic remodeling is a reduction in wall area (41, 59). Age-related cerebral artery remodeling could increase the risk of cerebrovascular accidents especially in situations where other risk factors, such as hypertension, are present (48). Therefore it is important to fully understand the effects of aging on cerebral artery structure.

The Stroke Treatment Academic Industry Roundtable (12a) recommendations for preclinical testing state that potential neuroprotective agents should be tested in aged animals. The effects of aging on peripheral arteries have been documented (25, 26). Aged atherosclerotic mice exhibit outward remodeling of the aorta compared with young mice (39). Artery stiffness increases with age in the rat aorta and small mesenteric arteries (27, 31). Hypertrophy of the artery wall has also been observed in small mesenteric arteries from aged rats (1, 27, 35). Aging also causes endothelial dysfunction in arteries from different vascular beds. Endothelial function is impaired in aorta, carotid, and basilar arteries from 18- and 24-mo-old mice (6, 10, 34). Interestingly, the basilar artery had the most impaired function and the authors attributed this to increased reactive oxygen species production and oxidative stress (34, 47). These studies suggest that the effects of aging on the peripheral and cerebral circulation are different; therefore we cannot assume that the effects of aging in the periphery will translate to the brain.

Cerebral artery autoregulation is an important mechanism to maintain cerebral blood flow within a normal range. The effects of aging on autoregulation are controversial. Recent studies in 24 mo C57Bl/6 mice show that aging impairs the ability of the cerebral arteries to autoregulate (52). This has also been observed in clinical studies (8). However, other studies suggest aging has no effect on autoregulation. A recent study in elderly people with mild cognitive impairment showed that low blood pressure was not associated with reduced cerebral blood flow (15). This suggests that in these patients autoregulation is normal. Similar findings have also been made in a younger population (54).

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Cerebral arterioles interact with neurons, astrocytes, and glial cells to form the neurovascular unit, which coordinates coupling between neural activity and local cerebral flow. Therefore cerebral arteries may behave differently from arteries in the peripheral circulation (28). The goal of our study was to characterize the effects of aging on the biomechanical properties of the posterior cerebral artery (PCA) and parenchymal arterioles (PAs) and to assess the differential effects of aging on the microcirculation and the large pial arteries. We hypothesized that aging would impair the biomechanical properties of the PCA and the PAs resulting in outward artery remodeling and increased artery stiffness. The PCA regulates blood flow and pressure to the posterior cerebral circulation. The PCAs arise from the basilar artery and supply the midbrain, basal nucleus, and thalamus, among other structures (22). The PCA is used as a model of a large pial artery. It is frequently studied in mice because it is more amenable to pressure myography studies than the middle cerebral artery (MCA), which is highly branched. The PAs arise from the pial arteries, via the penetrating arterioles, which are located in the Virchow-Robin space. PAs studied were branching off the MCA and were 1–2 mm downstream the Virchow-Robin space.

Fig. 1. Aging increases blood pressure with no change in heart rate. Data are means ± SE (n = 4 for Young and n = 3 for Old) of 24-h averages of blood pressure or heart rate, as indicated, measured by telemetry. Two-way ANOVA indicated significant effects of age for all blood pressures (A–D) (*P < 0.05), but not for heart rate (E) or activity (F) (P > 0.05). Blood pressure data recording started on day 3 after telemeter implantation.

Fig. 2. Night-time blood pressures are elevated in aged mice. Data are means ± SE (n = 4 for Young and n = 3 for Old) of 1-h averages of mean arterial pressure (MAP) for the last 8 days of the data shown in Fig. 1 showing substantially elevated night-time blood pressures in the aged mice. The 0-h time point represents the first midnight of the time period shown. Two-way ANOVA indicated significant effects of time and age, with a significant interaction term (P < 0.05 for each).
space; these arterioles are in direct contact with the brain parenchyma. Unlike the MCA or PCA, the PAs have few branches. The PAs play a critical role in controlling blood flow and pressure in the cerebral microcirculation and are important in determining overall cerebrovascular resistance (12). PAs are composed of endothelial cells and smooth muscle cells, but they are different from pial arteries and arteries in peripheral vascular beds in that they lack extrinsic innervation (9).

MATERIALS AND METHODS

Animal model. All experimental protocols were approved by the Michigan State University Animal Care and Use Committee and were performed in accordance with the American Physiological Society’s Guiding Principles in the Care and Use of Animals. Male C57Bl/6 mice purchased from the National Institute of Aging at Charles River Laboratories were housed on 12:12-h light/dark cycle with food and water ad libitum. Mice were studied at 3–5 mo (young) and 22–24 mo (old) of age. For pressure myography studies, mice were euthanized by CO2 asphyxiation followed by cervical dislocation and decapitation.

Telemetry. Blood pressure was measured by telemetry as described previously (62). Mice were anesthetized with 3% isoflurane/1% oxygen for implantation of a catheter attached to a radio telemetry transmitter (Data Sciences International, St Paul, MN) in the abdominal aorta via the femoral artery; the transmitter body was placed subcutaneously. Mice were allowed to recover for 3 days and then mean, systolic, and diastolic arterial pressures were measured continuously (10 s averages collected every 10 min, 24 h/day). Data were expressed as the 1- or 24-h averages of systolic, diastolic, mean arterial pressure, and pulse pressure (systolic pressure – diastolic pressure). We report the latter because it is an independent risk factor for cardiovascular disease.

Pressure myography. The brain was collected at euthanasia and the biomechanical properties of isolated PCA and PAs were assessed by pressure myography as described previously (42–45). To dissect the PAs, a 5 × 3 mm section of brain containing the MCA was isolated. Then the pia with the MCA was separated from the brain and the PAs branching from the MCA were used for experiments (43). The PAs we studied were branching off the MCA and were located 1–2 mm downstream the Virchow-Robin space. PCAs and PAs were mounted between two glass micropipettes in a custom-made cannulation chamber (61). A servo-null system was used to pressurize the arteries and arterioles. Arteries and arterioles were equilibrated in physiological salt solution (PSS) containing (in mM) 141.9 NaCl, 4.7 KCl, 1.12 KH2PO4, 1.7 MgSO4·7H2O, 10 HEPES, and 5 dextrose under zero-flow conditions at 37°C. Ethylene glycol tetraacetic acid (EGTA; 2 mM) and sodium nitroprusside (SNP; 10⁻⁵ M) were added to maintain the smooth muscle in a relaxed state. A leak-test was performed prior to each experiment; any artery that could not maintain its intraluminal pressure (60 mmHg for the PCA and 40 mmHg for the PAs) was discarded. A pressure-response curve was constructed by...
increasing the intraluminal pressure from 0 to 120 mmHg at 20-
mmHg increments. The PCAs and PAs were equilibrated at each
pressure for 5 min, then lumen and outer diameters were measured
using a 10X objective (Nikon Plan objective; Numerical Aperture: 0.25) with
a Nikon Eclipse TS100 microscope. The average of the outer and lumen
diameter at each pressure was recorded using MyoVIEW II 2.0
software (Danish Myo Technology, Aarhus, Denmark). These
measures were compared and used to calculate wall thickness (outer
diameter – lumen diameter). Wall cross-sectional area was calculated
using the formula “artery area = (π/4) × (outer diameter × outer diameter – lumen diameter)”. Wall area, wall stress, strain, and distensibility were calculated as described previously (4). Wall stiffness was quantified using the β-coefficient calculated from the individual stress-strain curves using the model
(y = ae^bx), where y is wall stress, x is wall strain, a is the intercept and
β is the slope of the exponential fit; a higher β-coefficient represents
a stiffer vessel.

Immunofluorescence. Quantification of artery and capillary numbers
in young and aged mice was performed by immunofluorescence
(IF) staining of the endothelial cell marker isoelectin GS-IB1. Mice
were transcardially perfused with 100 ml of PSS containing 2.8 mM
calcium plus 1,000 UI/ml heparin sodium salt, 10^{-5} M SNP, and 10^{-5}
M diltiazem to maximally dilate the cerebral vasculature. The perfu-
sion pressure was maintained at 60 mmHg. Following perfusion with
PSS, mice were perfusion-fixed with 60 ml of 4% formaldehyde.
Brains were then washed twice in 0.01 M PBS (0.05 M) and placed in 20% sucrose-PBS solution for cryosec-
tioning. Cryosections (20 μm thick) were incubated overnight in
0.01 mg/ml isolectin GS-IB4 Alexa Fluo-568 conjugate (Invitro-
gen, Cambridge, CA) at 4°C. This is a conjugated lectin; therefore
incubation with a secondary antibody was not necessary. The next
day, sections were washed 4X in 0.01 M PBS (5 min each wash)
and coverslips were mounted using Prolong antifade reagent (In-
vitrogen, Carlsbad, CA) (21). Two fields of the premotor cortex,
one in each hemisphere, more specifically in the second and third
layers of the neocortex, were acquired using a 20X objective
(UPLSAPO 20X NA: 0.75) coupled to an inverted Olympus
Confocal Laser Scanning microscope (Olympus America, Central
Valley, PA) with Olympus Fluoview FV1000 (Olympus America).
All images were acquired using the red fluorescent dye Alexa Fluor
568 that has an excitation wavelength of 578 nm and an emission
wavelength of 603 nm. Sections without the isoelectin served as
negative controls. For the quantification of the vessel density 3D
volume reconstruction of the z-stacks were made. We rotated the
3D volume reconstruction to better visualize when a vessel started
and where it ended to make sure we were not counting the same
vessel twice. We also used a grid to make sure we were counting
the vessel correctly and not twice. We did not have software
available to do the quantification of the vessels; therefore all the
quantifications were done manually by the investigator using
ImageJ (46).

Calcium and collagen staining. The Investigative Histology Lab-
oratory at Michigan State University performed staining for calcium
and collagen in the cerebral arteries. Calcification of the intraparen-
chymal arteries was assessed using the Von Kossa stain (33). Six
random fields were acquired to count the number of positive vessels
that contained calcium deposits. Masson’s Trichrome stain was used
to stain collagen in the cerebral arteries (7). Six fields were acquired
to quantify the amount of collagen deposition in the vessels. Images
were acquired using an Axioskop 40 (Carl Zeiss, Gottingen, Ger-
many) coupled to a camera (AxioCam MRc5 Carl Zeiss) with the
AxioVision Rel 4.6 software (Carl Zeiss Imaging Solutions, Gottingen,
Germany). A blinded investigator analyzed images.

Statistical analyses. All data are presented as means ± SE. Body
weight, blood pressure, calcium and collagen deposition, and vessel
quantification data were analyzed by Student’s t-test. For analysis of
artery structure, two-way analysis of variance was utilized followed
by Bonferroni t-test for post hoc comparison of the means. All
statistical analyses were performed using GraphPad Prism 6.0 soft-
ware (GraphPad, San Diego, CA). In all cases statistical significance
was denoted by P < 0.05.

Drugs and chemicals. All drugs and chemicals were purchased
from Sigma Aldrich (St. Louis, MO) unless otherwise stated.

Fig. 4. Posterior cerebral artery (PCA) mechanical
properties were changed with aging. Wall
stress was increased (A) with age. Aging also
decreased strain in the PCA (C). The PCA was
less distensible with age (B). Wall stress vs.
strain was reduced in the older mice (D). Data
are presented as mean ± SE; ***P < 0.001,
**P < 0.01, *P < 0.05, 2-way ANOVA fol-
lowed by Bonferroni for post hoc comparisons.
Data from Fig. 3 were used to calculate the
mechanical properties of the PCA.
RESULTS

Physiological measures. Old mice were significantly heavier than the young mice (31.02 ± 1.57 vs. 34.79 ± 1.09 g; young vs. old). Blood pressure, measured by telemetry, showed that in our cohort of mice, advanced age was associated with higher systolic, diastolic, mean, and pulse pressures (Fig. 1, A–D). However, we observed no significant effect of age on heart rate or activity (Fig. 1, E and F). The higher blood pressure in older mice resulted mainly from substantial differences in blood pressure during the night-time when the animals are most active (Fig. 2).

Biomechanical properties of the posterior cerebral artery and penetrating arterioles. Older mice had increased PCA outer and lumen diameter (Fig. 3, A and B). Lumen cross-sectional area was also larger in the old mice (Fig. 3C). No significant differences between young and old mice were observed in the wall thickness and cross-sectional area (Fig. 3, D and E). Older mice showed decreased wall-to-lumen ratio (Fig. 3F). The mechanical properties of the arteries differed with age. Wall stress was higher in old mice (Fig. 4A) while wall strain, distensibility, and stress vs. strain were lower in PCAs from old mice (Fig. 4, B–D).

Aging was also associated with PA remodeling (Figs. 5 and 6). Wall thickness, cross-sectional area, and wall-to-lumen ratio were larger in PAs from old mice compared with young. No other significant differences in artery structure were observed. Older mice had greater wall stiffness in the PCAs (Fig. 7A) but not the PAs (Fig. 7B).

We compared arterial stiffening between the PCA and PAs in young and old mice separately. In the young mice, the PAs were stiffer than the PCAs (Fig. 7C). No differences in stiffness were observed between the PCAs and PAs from the old mice (Fig. 7D).

Calcium and collagen in the arterial wall. In a small cohort of mice, we observed that older mice did not have a significantly greater number of cerebral arteries with increased calcium deposits in the wall (P = 0.3) (Fig. 8A). However, as shown in the representative images, it appears that the percentage of calcification in the individual cerebral arteries from old mice is greater (Fig. 8, C and D). Arteries from old mice had more collagen deposition (Fig. 9A). Representative images are shown (Fig. 9, B–D).

Artery and capillary density in the cerebral cortex. Artery and capillary density was quantified using the endothelial cell marker Isolectin IB-4. Two fields of the neocortex, one in each hemisphere, were acquired. In a small cohort of mice, we

![Fig. 5. Aging increases wall thickness in the penetrating arterioles. Outer diameter (A), lumen diameter (B), and lumen area (C) of the penetrating arterioles were not significantly changed with aging. Wall cross-sectional area (D), wall thickness (E), and wall-to-lumen ratio (F) were increased with age. Data are presented as means ± SE. ***P < 0.001, *P < 0.05, 2-way ANOVA.](attachment:image.png)
observed that old mice had significantly fewer arteries and capillaries in the cerebral cortex (Fig. 10).

**DISCUSSION**

The novel finding of our study is that aging is associated with changes in the biomechanical properties of the PCA and PAs. The effects of aging on the biomechanical properties of the posterior cerebral circulation and smaller cerebral arterioles have not been widely characterized (29). The PCA was utilized as a model of a large pial artery. The PCA is important for regulating the blood flow to the posterior cerebral circulation. The PAs serve as a bottleneck for perfusion of the neocortex (41). PAs also play an important role in determining the outcome of ischemia; however, these arterioles have not been well characterized (9, 21, 43) and the effects of aging have not been assessed. In the PCAs, aging was associated with an increase in the outer and lumen diameter and a decrease in wall-to-lumen ratio. Aging was also associated with increased wall stress and stiffness. However, wall strain and distensibility were decreased with age in the PCAs. In the PAs, no changes in the size of the artery were observed but aging was associated with changes to the wall structure. The wall area, wall thick-

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**Fig. 6.** Aging resulted in changes to the mechanical properties of the penetrating arterioles. At 120 mmHg, wall stress was increased with age (A). Wall strain (C), distensibility (B), and stress-strain (D) were unchanged. Data are presented as means ± SE. *P < 0.05, 2-way ANOVA followed by Bonferroni for post hoc comparisons. Data from Fig. 5 were used to calculate the mechanical properties of the PCA.

**Fig. 7.** Aging increases vascular stiffness in the posterior cerebral artery, and vascular stiffness is different depending on the type of artery. Wall stiffness is increased with aging in the posterior cerebral artery (A) but not in the penetrating arterioles (B). An increased β-coefficient represented increased wall stiffness. In young mice vascular stiffness is increased in the small arteries compared with large arteries (C). This was not the case in old mice (D). PA, parenchymal arterioles. Data are presented as means ± SE. *P < 0.05, **P < 0.01, Student’s t-test. Data from Fig. 4 and 7 were used to calculate the mechanical properties of the PCA.
ness, and wall-to-lumen ratio of the PAs were increased with age while wall stress was reduced.

The increase in the lumen diameter of the PCA we observed with age could increase cerebral blood flow and cause hyperemia. This could be compensated for by increased myogenic tone. We did not measure myogenic tone in this study, but studies in aged mice treated with angiotensin II show age, combined with hypertension, causes a loss of myogenic tone and autoregulation in the MCA (52). Loss of myogenic tone in a large artery such as the MCA increases the risk of rupture of the PAs with fluctuations in blood pressure. In the PAs, the increased wall thickness without changes in wall stress we observed could be a positive adaptation to protect these arterioles from rupture and vascular damage.

One of the strengths of our study is the advanced age of the mice in the aged group. At 24 mo old the mice used in this study were close to the end of their natural lifespan; therefore these mice truly model the geriatric population. The use of telemetric blood pressure recording in this study also is a strength because it is a more accurate technique to measure blood pressure than tail-cuff plethysmography. We also avoided the carotid catheterization approach used in many mouse telemetry studies because it may artificially alter blood pressure by affecting baroreflex function. Our studies show that mean arterial pressure and pulse pressure were increased with age. This is in contrast to studies using tail-cuff plethysmography which suggest that aging does not affect (52) or reduces (19) blood pressure. However, our studies are in agreement with clinical studies showing that blood pressure increases with age (20, 40, 58, 55). Pulse pressure was markedly increased in the aged mice; this could lead to vascular cognitive impairment (17, 32, 56).

The higher day-night blood pressure ratio that we observed in older mice (Fig. 2) is notable because this ratio is known to be an independent predictor of all-cause mortality and cardiovascular events in humans even after adjustment for 24 h average blood pressure (11). A caveat to our study is that we do not know when blood pressure became elevated in the aged mice because blood pressure was only measured at the 24-mo time point. Age alters the ability of cerebral arteries to adapt to hypertension. Cerebral arteries from young mice have the ability to functionally and structurally adapt to hypertension (38, 52). However, the MCAs from 24-mo-old mice have an impaired ability to respond to hypertension (51). Our studies show that age results in high blood pressure. Therefore, it is possible that the ability of the PCA and PAs from old mice to adapt to hypertension is impaired. It is also important to note that in our study we observed outward remodeling which is the opposite of what we would expect with hypertension (41). It should be noted that angiotensin II-induced hypertension is likely to have a more rapid onset than an aging-associated blood pressure change; with a more gradual increase in blood pressure the mechanisms of artery remodeling may be different.

Our preliminary studies of a small cohort of mice suggest that the ageing process also resulted in artery rarefaction, that is, a decrease in the vessel density in the brain. Cerebral artery rarefaction has been observed in some models of hypertension (37, 49) and aging (52). A reduction in the number of vessels in the brain could lead to chronic hypoperfusion (28). We also

Fig. 8. Aging may increase calcium content in the wall of cerebral arteries. In a small cohort of mice, arteries with calcium deposits were counted in young and old mice. The amount of vessel with calcium deposits or the increase in the percentage of calcification (data not shown) was not significantly different between both groups (A). Representative images at 40× magnification are shown (B: positive control; C: young mouse; D: old mouse). The arrows indicate the arteries. Data are presented as means ± SE. P = 0.3 by Student’s t-test; n = 3.

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show that aging increases pulse pressure and this has been associated with artery rarefaction (50, 52). However, we do not know if the changes in PCA and PA structure observed are a causative factor in the artery rarefaction or if remodeling and rarefaction occur independent of each other.

We observed increased stiffness in the PCA but not in the PAs, suggesting that age-associated changes in stiffness are different in small arterioles and large cerebral arteries. The increases in stiffness in the PCA could have been a result of higher mean arterial pressure or pulse pressure in the older mice rather than aging per se. In rat models of essential hypertension, the large cerebral arteries remodel first; this presumably serves to protect the smaller downstream arteries from the increased pressure (28). The small arteries remodel after a prolonged period of hypertension (28). It is possible that the same pattern of remodeling occurs with aging and that the cerebral artery remodeling we observed was a consequence of both aging and increased blood pressure. Further studies will be required to determine if cerebral artery stiffness in the aged mice is caused by aging itself or by increased blood pressure. Increased arterial stiffness is a hallmark of artery dysfunction and an independent predictor of cardiovascular disease (16). In peripheral arteries, aging has been associated with changes in the composition and organization of the arterial wall that increase artery stiffness (13). We observed an increase in stiffness of the PCA without an increase in wall thickness suggesting that increased stiffness is the result of changes in extracellular matrix composition.

Collagen and elastin are important components of the extracellular matrix (14), and they play key roles in maintaining the strength and elasticity of the arterial wall. With normal aging, collagen and elastin expression is differentially regulated (13) such that the increased stiffness in large arteries is associated with increased collagen and reduced elastin deposition (30, 53). The increase in collagen deposition could alter the mechanical properties of the artery wall resulting in stiffening. Mandala et al. (29) showed that in adult normotensive rats the amount of elastin in the PCA was reduced compared with young rats but no changes in collagen were observed. Our findings suggest that collagen deposition is increased with aging in the penetrating arterioles. The discrepancy between these studies can be attributed to the age and strain of the animals; Mandala et al. studied 11- to 12-mo-old Sprague Dawley rats, while we studied mice that better represent the geriatric population (22–24 mo old). The elastin fragmentation that occurs with increased age is associated with increased expression of the matrix metalloproteases (MMP) (18, 53, 57). MMP-2 and -9 in particular have been associated with elastin calcification (2, 3). The ageing process also increases the amount of calcium and phosphate in the wall (63, 64). This is associated with the calcification of the elastic fibers and could contribute to artery wall stiffness. We found that the amount of calcium in the wall and the number of arteries with calcium deposits might be increased with ageing but our data did not reach statistical significance. We recognize that these studies

Fig. 9. Aging increases the percent of collagen deposition in cerebral arteries. In a small cohort of mice, the percentage of collagen deposits in the wall of young and old mice was quantified. Aging resulted in a significant increase in collagen deposition (A). Representative images at 40× magnification are shown (B: positive control; C: young mouse; D: old mouse). The arrows indicate the artery. An artery with increase collagen content will be stained purple. Data are presented as means ± SE. ****P < 0.001 by Student’s t-test; n = 3.
were underpowered (\(n = 3\)) but the number of aged mice was limited; therefore further studies will be required.

Liu et al. (28) showed that aged mice have smaller infarct size after an ischemic stroke than young mice. Interestingly, however, the functional impairments in old mice post-stroke were much worse. Post-stroke the cerebral arteries are maximally dilated such that flow is proportional to the lumen diameter. Our studies showed that advancing age is associated with increased lumen and artery cross-sectional area of the PCA. This could be the cause of the smaller infarct observed in aged rats. The magnitude of the increase between the outer and lumen diameter was different. The change with aging was greater for the outer diameter of the PCA and this is likely the cause of the increase in artery stiffness. Wall thickness of the PCA was not changed with age but it was increased in the PAs. This difference implies that missing protection from high intraluminal pressure in pial arteries is associated with increased wall thickness of the downstream arterioles. In the PAs, wall thickening with age is probably a result of smooth muscle cell hypertrophy (30). Our results are consistent with previous findings showing that in human conduit arteries such as the aorta, iliac arteries and carotid arteries, aging is associated with increased lumen area and wall thickening (53). Our studies also show that aged mice have higher wall cross-sectional area in the PCA and PAs than do young mice. In contrast, in aged Fischer rats the wall cross-sectional area of the pial arteries was less than in young animals (19). Aging also altered the mechanical properties of the PCA resulting in a less distensible artery and decreased wall strain. This is consistent with the work of Hadju et al. (19), in the pial arteries of 24-mo-old Fischer rats. However, in another study the distensibility of the MCA from 24-mo-old mice was not changed (50). The reduced distensibility we observed may be also associated with alterations of the artery stiffness.

A limitation to our study is that we did not study the development of spontaneous myogenic tone or endothelial dysfunction with age. However, it is known that aging is associated with endothelial dysfunction in the basilar artery through a reactive oxygen species-dependent mechanism (34, 47). Aging also impairs the ability of the MCA to generate tone in response to static (52) and pulsatile pressure (50). Another limitation of our studies is that we did not assess the mechanism of artery remodeling; this is a topic for future study. Possible mechanisms involve changes in the arrangement of the smooth muscle cells, increases in the expression of MMPs such as MMP-2 and -9, and elastin fragmentation. We did show that the cerebral arteries of aged mice have increased collagen that could have an important role in the changes in the artery wall observed.

In summary, aging is associated with structural changes that increase the wall stiffness of the PCA and wall stress and wall thickness of the PAs; combined, these changes could result in a dysregulation of cerebral blood flow that would increase the risk of stroke and dementia. The vasculature is a potential therapeutic target for stroke, and potential neuroprotective or neurorestorative therapies need a functioning vasculature to deliver the drug to the site of injury. Therefore, it is important to fully understand the mechanisms of age-associated cerebral

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**Fig. 10. Aging decreases artery density.** In a small cohort of mice, the amount of arteries and capillary number was quantified using Isoelectin-IB4. In each animal, two images were acquired in the neocortex, one per hemisphere. Representative images are shown above. B: control; C: young; D: old. Data are presented as means ± SE. *\(P < 0.05\), Student’s \(t\)-test; \(n = 3\).
artery remodeling to improve cerebrovascular health. For practical reasons all of our studies were conducted in male mice; therefore, further studies should be conducted in female mice to evaluate sex differences. In future studies we should assess if aging impairs vascular tone of the cerebral arteries and if the age-associated changes in cerebral artery structure are caused by the increased blood pressure or aging itself.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


