Effects of Aging on Cerebral Vasospasm After Subarachnoid Hemorrhage in Rabbits

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Background and Purpose—The effects of aging on cerebral vasospasm after subarachnoid hemorrhage (SAH) remain to be elucidated. The aim of this study was to clarify age-related differences of vasospasm and of papaverine reactivity in the responses of basilar arteries after SAH in rabbits.

Methods—Rabbits receiving a single injection of arterial blood into the cisterna magna were divided into 3 groups: young (2 to 3 months old), adult (6 to 9 months old), and old (20 to 40 months old). Vertebrobasilar angiograms were obtained before SAH and 1, 2, 4, and 7 days after SAH. Papaverine was administrated selectively via the vertebral artery on day 2, and serial angiography was performed for up to 2 hours. Vessel structures were assessed with light microscopy on days 1, 2, 4, and 7 after SAH and at 10, 30, and 60 minutes after papaverine infusion.

Results—Mortality from SAH in old rabbits was 40%, whereas that of young and adult rabbits was 0%. Angiograms revealed that SAH induced maximal constriction of the basilar arteries on day 2 in all age groups, and the constrictions were significantly increased with age at all time points investigated. The degree of dilatation of spastic basilar arteries after intra-arterial papaverine administration significantly decreased with age. Duration of the efficacy of papaverine became significantly shorter with age. Vessel diameter returned to the preinfusion value 120, 60, and 30 minutes after infusion in young, adult, and old rabbits, respectively. Light microscopy in old rabbits showed luminal narrowing and corrugation of the internal elastic lamina not only in the basilar arteries but also in small arteries and intraparenchymal arterioles.

Conclusions—This study suggests that aging increases the degree of vasospasm in rabbits. The impaired reactivity to papaverine with aging might imply the early transition of the aged vessel to the papaverine-resistant chronic stage.

Key Words: aged ▪ aging ▪ angiography, digital subtraction ▪ cerebral ischemia, transient ▪ rabbits

Aging is a major risk factor for poor outcome in patients with aneurysmal subarachnoid hemorrhage (SAH). Recent studies have shown that the incidence of SAH in the elderly, especially those older than 60 years of age, is increasing with the increased age of the general population and that earlier surgical intervention, including endovascular therapy, is indicated for those elderly patients with SAH.

Cerebral vasospasm is the major cause of morbidity and mortality among patients operated on at early or delayed intervals after SAH. Lanzino et al. in a large study, reported that the incidence of symptomatic vasospasm increased with advancing age, although the incidence of asymptomatic vasospasm was lower in elderly than in younger patients. There is, however, controversy over the effects of aging on severity of vasospasm. Contractile responses of cerebral arteries to vasoconstricting agents have been shown to be potentiated with age, atherosclerosis, hypertension, and hypercholesterolemia. Thus, aging may affect the severity of vasospasm in which the pathogenesis is considered multifactorial. Therefore, it is crucial to know the effect of aging on cerebral vasospasm. However, there have been no experimental studies that have analyzed the relationship between age and vasospasm in vivo.

More recently, issues regarding intra-arterial infusion of papaverine for the treatment of vasospasm have been discussed, such as short duration of the effect, increasing intracranial pressure, transient neurological deficits, mydriasis, transient thrombocytopenia, and respiratory depression associated with vertebrobasilar infusion. Papaverine is a potent, non–endothelium-dependent vasodilator and a nonspecific inhibitor of phosphodiesterase in vascular smooth muscle. It has been reported that vasoconstricting responses induced by papaverine in intact vessels of the elderly are essentially unchanged. Several authors have reported that there are papaverine-sensitive and papaverine-resistant phases during the time course of vasospasm and that revers-
ibility of vasospasm by papaverine depends on the period after SAH and on the severity of vasospasm.

Thus, the present study was designed to clarify age-related differences in vasospasm, as well as the efficacy of papaverine in relation to the responses of basilar arteries after SAH.

Materials and Methods

Animal Preparation

All procedures were approved by the Animal Care and Use Committee of Okayama University and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

One hundred twenty-seven male New Zealand White (NZW) rabbits weighing 1.7 to 4.5 kg each were used for this study; 45 were classified as young (2 to 3 months), 30 were adult (6 to 9 months), and 52 were old (20 to 40 months). All animals were housed singly at our institution with free access to food and water. Animals were anesthetized with ketamine hydrochloride (40 mg/kg IM) and pentobarbital sodium (20 mg/kg IV) supplemented at 5 to 10 mg · kg⁻¹ · h⁻¹ via ear vein. Spontaneous respiration was permitted throughout the experiment. The femoral artery was exposed by sterile technique, and a catheter was placed to measure systemic blood pressure and heart rate and to obtain arterial blood. Arterial blood gases were monitored and maintained within normal levels throughout the experiment. The PaCO₂ was adjusted to be maintained at 35 to 40 mm Hg by supplemental anesthetic. Body temperature was maintained between 37°C and 38°C with a heating pad.

Experimental Design

All animals underwent baseline cerebral angiography 3 to 6 days before induction of SAH. Three rabbits in each group served as controls. The rabbits subjected to SAH were randomly assigned to 4 groups of different ages after SAH: day 1 (10 young, 4 adult, and 10 old), day 2 (18 young, 9 adult, and 20 old), day 4 (10 young, 10 adult, and 10 old), and day 7 (4 young, 4 adult, and 9 old). Angiography was repeated on days 1, 2, 4, and 7. Serial angiograms before SAH and on days 1, 2, 4, and 7 were also obtained in the same rabbits (3 young and 3 old). After completion of angiography, animals were killed at each time point after SAH, except for 33 surviving day 2 rabbits, which were used for papaverine infusion. Perfusion fixation was performed in each SAH animal and in control animals for histological analysis.

Papaverine (5 mg/kg; 15 young, 6 adult, and 12 old rabbits) diluted to 3 mL of normal saline was manually infused over 5 minutes via the left vertebral artery on day 2. Serial angiography was repeated over the subsequent 2 hours to monitor the arterial caliber. The concentrations of papaverine used in the present study ranged from 0.28% to 0.66%, which was within recommended concentrations without toxicity to the arterial wall.29 In all, 9 young and 6 old rabbits were killed at 10, 30, and 180 minutes and at 10 and 30 minutes after infusion, respectively, when the basilar artery diameter returned to preinfusion value. Perfusion fixation was performed at each time point.

Angiography and Induction of SAH

The anesthetized rabbits were placed in a supine position, and a 4F angiocatheter was introduced through the exposed femoral artery. Then the catheter was advanced into the left vertebral artery, and vertebrobasilar angiograms were obtained by manual injection of 0.2 to 0.4 mL of nonionic contrast medium. A magnification standard of 1-mm width of the lead string was included in each angiogram.

The anesthetized rabbits were placed in a prone position with the nose ~30° below horizontal. With the use of aseptic techniques, a midline nuchal incision was made, and muscles were cleared to expose the atlanto-occipital membrane. The cisterna magna was punctured by a 24-gauge butterfly needle. One to 1.5 mL of cerebrospinal fluid was withdrawn, and 1 mL/kg fresh nonheparinized autologous arterial blood that had been taken from the central ear artery was injected over 5 minutes. The needle was withdrawn, and nuchal muscles and skin were closed with sutures. The animals were then placed in a head-down position for 20 minutes to facilitate blood settling around the basilar artery.

Analysis of Angiograms

The basilar artery diameter was measured at 3 points: 1 mm proximal to the basilar bifurcation, the midportion of the basilar artery, and 1 mm distal to the vertebrobasilar junction.23 All angiograms were corrected for variations using the magnification standard in each radiograph. Each artery diameter at the 3 points was averaged for the final value. The basilar artery diameter was expressed as a percentage of the pre-SAH diameter.

Histological Study

After the rabbits were anesthetized and killed, perfusion fixation was performed transcardially with 200 mL of physiological phosphate buffer solution at 37°C, followed by 500 mL of a solution of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4) at a perfusion pressure of 1500 mm H₂O. The brain was removed, immersed in fixative, and stored at 4°C for a minimum of 1 week. Sections of 4.5-μm thickness were cut on an ultramicrotome, mounted on glass slides, and stained with elastica van Gieson for light microscopy.

Statistical Analysis

All values are expressed as mean±SEM. Student’s t tests were used to compare physiological variables. To compare the degree of vasospasm within groups over time and between groups at each time, ANOVA was performed, followed by Scheffé’s F test for multiple comparisons. For comparisons between groups after papaverine infusion, a 2-way ANOVA was performed, with age and time as the 2 factors of analysis. Different age groups were then compared at each time, with probability value adjusted by Fisher’s protected least significant difference method. A value of P<0.05 was considered significant.

Results

Among animals subjected to SAH, none of the young or adult rabbits died before the post-SAH angiography and papaverine infusion study. In contrast, 21 of the 52 old rabbits died before the experiments, due not to procedure-related complications but to the insult caused by SAH. Eighteen (86%) of 21 rabbits died within 24 hours and 3 (14%) died within 48 hours. Mortality of the old rabbits from SAH was 40%. SAH produced focal neurological signs only in 2 (6%) of the surviving 31 old rabbits. One had hemiparesis and the other showed transient apathy and involuntary movements. Both rabbits were older than 3 years of age at the time of experiment.

Physiological Variables

Physiological variables for intact rabbits are shown in the Table. Body weight was significantly higher in adult and old rabbits than in young rabbits (P<0.01). Mean arterial pressure was significantly higher in adult rabbits than in young or old rabbits (P<0.05). Pulse pressure was significantly higher in old rabbits than in young and adult rabbits (P<0.05). Heart rate and arterial PaCO₂ were not significantly different among the groups. Angiographic pre-SAH lumen diameter of the basilar artery was significantly larger in adult and old rabbits than in young rabbits (P<0.05).

Regarding papaverine infusion, other than mean arterial pressure, there were no significant differences in variables among groups (data not shown). Mean arterial pressure was
The constrictions were significantly increased with aging at all time points investigated. Values are mean ± SEM. *P<0.01 vs young; †P<0.01 vs adult; ‡P<0.05 vs adult.

The magnitude of vasospasm showed maximal constriction of the basilar artery on day 2 in all groups (61±2%, 52±2%, and 44±2% in young, adult, and old rabbits, respectively; Figure 1). In young rabbits, arterial caliper returned almost to the pre-SAH baseline value on day 7 (93±4%), whereas in adult and old rabbits, it showed no significant difference between day 2 and day 7 (61±2% and 48±6% in adult and old rabbits, respectively).

The magnitude of vasospasm showed increasing severity with advancing age at all time points investigated. A significant difference was observed between young and old rabbits at day 1 (P<0.01), among all groups at day 2 and day 4 (P<0.05), and between young and adult rabbits (P<0.01) and young and old rabbits (P<0.01) at day 7. Representative serial angiograms before SAH and on days 1, 2, 4, and 7 in a single rabbit from the young group and a single rabbit from the old group are shown in Figure 2.

Papaverine Infusion

During intra-arterial infusion of papaverine, transient respiratory depression, including respiratory arrest, was frequently observed. The magnitude of vasospasm showed significant relaxation induced by papaverine in all groups (P<0.001), but the degree of relaxation decreased with advancing age (Figure 5). Duration of the efficacy of papaverine was transient in all groups and decreased with advancing age. Vessel diameter returned to the preinfusion value in ~120, 60, and 30 minutes after infusion in young, adult, and old rabbits, respectively. Representative serial angiograms after papaverine infusion in the same young rabbit and the same old rabbit are shown in Figure 6.

Histopathological Findings

Changes After SAH

In young rabbits, corrugation of the internal elastic lamina (IEL) was observed on day 2 and day 4 and resolved on day 7 (Figure 3). Meanwhile, in old rabbits, corrugation of the IEL was observed on days 1, 2, 4, and 7. The corrugation of IEL appeared earlier and persisted longer in old than in young rabbits. The degree of corrugation became more severe with advancing age. Thickening of the media and adventitia was greater in old than in young rabbits at all time points investigated. Adult rabbits showed a similar trend to that of old rabbits (data not shown). Currugation of IEL and thickening of the media and adventitia were observed in small arteries and intraparenchymal arterioles in old rabbits but not in young rabbits (Figure 4).

Changes After Papaverine Infusion

In young rabbits, corrugation of the IEL decreased within 10 minutes after infusion and disappeared within 30 minutes (Figure 7). Thinning of the media was observed within 10 minutes and was maintained at 30 minutes. At 180 minutes, thickening of the media and corrugation of the IEL recurred, and this finding was consistent with that of angiography. Meanwhile, in old rabbits, corrugation of the IEL and thickening of the media were already observed at 10 and 30 minutes after infusion.

Discussion

This is the first study to examine the in vivo effects of aging on cerebral vasospasm and on cerebral vascular responses to intra-arterial infusion of papaverine after SAH. There are 4 major new findings in this study. First, a high mortality rate due to initial hemorrhage was noted in older rabbits. Second, the degree of vasospasm was augmented and the resolution of vasospasm after maximal constriction was impaired more strongly with advancing age. Third, small arteries were more susceptible to vasospasm in old rabbits than in young and adult animals. Fourth, vasodilator responses to intra-arterial infusion of papaverine were impaired with aging.

Hemodynamic Parameter

Several studies have shown that animals become hypertensive with age.30,31 NZW rabbits used in this study had a tendency for mean arterial pressure to rise in adult animals, but this subsided in older animals. A similar trend has been reported with aging in rabbits,32,33 rats,34 and humans.35 Pulse pressure was significantly increased in old rabbits, as described previously.33 Therefore, increased pulse pressure in
old rabbits may influence vascular structure and reactivity after SAH.

**Vasospasm and Aging**

Previous clinical studies have suggested that the severity of angiographic vasospasm is not affected by age or that it is less severe in elderly patients and that the incidence of symptomatic vasospasm is lower, similar, or higher with advancing age. In the present study, constricting responses of the basilar arteries exposed to SAH increased with aging at all time points investigated. In addition, the resolution of vasospasm after maximal constriction on day 2 was impaired with aging. In particular, old rabbits showed severe and continuous vasospasm with nearly maximal constriction even on day 7, although a rabbit model of SAH with single injection of arterial blood into the cisterna magna usually represents a mild to moderate vasospasm of the basilar artery, the diameter of which returns to the pre-SAH level within a week, as previously demonstrated in a similar fashion in a canine and a rabbit single-SAH.

**Figure 2.** Vertebrobasilar angiograms after SAH in a single young rabbit (A, B, C, and D) and a single old rabbit (E, F, G, and H). Angiograms of young rabbit showed partial narrowing of the basilar artery on day 1 (B), maximum constriction on day 2 (C), and complete resolution of arterial narrowing on day 7 (D). Angiograms of old rabbit showed diffuse narrowing of the basilar artery on day 1 (F), maximum constriction on day 2 (G), and persistence of constriction even on day 7 (H).

**Figure 3.** Photomicrographs of representative cross sections of the basilar arteries of young (A, B, C, D, and E) and old (F, G, H, I, and J) rabbits at each time point after SAH, stained with elastica van Gieson. Young rabbits showed corrugation of the IEL on day 2 (C) and day 4 (D) and resolution of this change on day 7 (E). Old rabbits showed corrugation of the IEL and thickening of the media and adventitia on days 1 (G), 2 (H), and 4 (I). Mild corrugation persisted on day 7 (J). Magnification ×600 for all photographs.
model. This pattern in old rabbits is similar to that observed in a canine double-SAH model, which is considered an established SAH model with severe vasospasm that lasts for 14 days, suggesting that the rabbit basilar arteries exposed to SAH are vulnerable to vasospasm with aging.

In contrast to vasospasm in large arteries with impaired endothelium-dependent relaxations, it has been suggested that small arteries and intraparenchymal arterioles are resistant to vasospasm after SAH because of the preserved endothelium-dependent relaxations and a pial barrier that prevents erythrocytes in the subarachnoid spaces from entering into the perivascular spaces surrounding the penetrating arterioles. These findings are in agreement with the clinical findings of SAH in humans. Symptomatic vasospasm closely correlates with the narrowing of the major cerebral arteries and is usually reversible with timely transluminal balloon angioplasty. However, recent studies using the microvascular corrosion cast technique suggested that constrictions of small arteries and intraparenchymal arterioles in rats and dogs occurred after SAH. In addition, morphological changes of intraparenchymal arterioles, such as tapered narrowing with external folding, decreased internal diameter of arterioles, and increased wall thickness, were reported. Also, human pial arteries ranging from 300 to 900 μm ID showed hyperresponsiveness to contractile agents and spontaneous contractile activity within 48 hours of SAH. In the present study, we have histologically confirmed the vasospasm of brain stem small arteries and intraparenchymal arterioles in old rabbits with symptomatic vasospasm. This finding is supported by previous reports that diffuse severe and peripheral vasospasm had serious effects on the clinical state and outcome of elderly patients, that severe cerebral infarction due to diffuse vasospasm was found more commonly in aged patients, and that severe generalized angiographic vasospasm was seen without an increase of mean flow velocities by transcranial Doppler ultrasound. Thus, augmented constricting responses of large as well as small arteries and of intraparenchymal arterioles may predispose the aged to delayed cerebral ischemia.
Regarding in vivo studies of cerebral vasospasm among various animals such as rats, rabbits, cats, pigs, dogs, and primates, the canine “2-hemorrhage” model is most frequently used, but the best model of vasospasm that accurately reflects human SAH seems to be the primate model.\textsuperscript{60} In an aging study, however, large animals such as primates and dogs are not suitable because of the limited number available, their high cost, and the difficulty in knowing the accurate age of the animals. As previously shown in a rabbit model, SAH with a single injection of arterial blood into the cisterna magna produced a maximal (30% to 40%) reduction in basilar artery diameter at 2 to 3 days after SAH,\textsuperscript{61,62} mimicking the situation observed in monkeys\textsuperscript{63} and humans.\textsuperscript{64,65} Thus, we consider rabbits to be the most suitable and largest in vivo aging model of SAH that closely reflects human SAH.

A limitation of the present in vivo study is that old rabbits used were 20 to 40 months of age without atheromatous changes, which represents 50 to 60 years of age in humans. In view of the definition of aged or senescence as the age at which mortality approximates 50%, we confirmed the caged-rabbit mortality rate as being >50% within 3 years of age in our institution. Thus, the old rabbits used in the present study are consistent with the term “the old.” It is likely that differences in responses between groups are related to differences in age, but we cannot exclude the possibility that other factors may also contribute.

**Efficacy of Papaverine and Aging**

It has been reported that the ability of papaverine to reverse vasospasm depends on the amount of time since SAH and on the severity of vasospasm.\textsuperscript{19,27,28} The present study demonstrated angiographically the reduced in vivo maximal vasodilating responses of the basilar arteries on day 2 to intra-arterial infusion of papaverine during aging and the decreased duration of the effect of papaverine with aging. These may be attributable to the increased severity of vasospasm with aging.

The magnitude of relaxation in young rabbit basilar arteries to papaverine was 100% of pre-SAH levels. This perfect resolution of vasospasm in the young rabbit by papaverine is consistent with a previous report using a rabbit model of SAH with gradual injection of an extremely large volume (6 mL in total) of arterial blood.\textsuperscript{27} That report indicated that there are 2 phases to arterial narrowing based on the in vivo relaxation responses to papaverine (an early, pharmacologically reversible phase and a later, irreversible phase) and that a papaverine-resistant phase emerges at day 3. In this sense, the adult and old rabbits in the present study demonstrated reduced maximal vasodilatation by papaverine on day 2, suggesting that they had already become resistant to papaverine on day 2 and that aging may accelerate the earlier transition of the vessel after SAH to the papaverine-resistant chronic stage.

![Figure 6. Vertebrobasilar angiograms after papaverine infusion on day 2 in young (A, B, C, D, and E) and old (F, G, H, and I) rabbits. Vessel dilatation in young rabbits continued for 30 minutes (D). Vessel diameter returned to the preinfusion value in 60 minutes (E). In old rabbits, vessel was less dilated within 5 minutes (H) and returned to the preinfusion level in 10 minutes (I).](http://stroke.ahajournals.org/)

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Speculations on the Mechanisms Involved and Implications

On the basis of previous studies and our own findings, several explanations can be suggested. With respect to the increased severity of vasospasm at the earlier stage after SAH during aging, one hypothesis could be an augmented contraction to vasoconstrictor agents. Previous studies have shown that vasoactive agents such as serotonin,\textsuperscript{9-11} endothelin-1,\textsuperscript{33} thromboxane,\textsuperscript{12-14,66} histamine,\textsuperscript{9,66} and prostaglandin E\textsubscript{2},\textsuperscript{12} all of which have been considered as possible spasmogens, increase the direct contractile effect with aging. In addition to the direct contracting effect, threshold concentrations of endothelin-1 potentiate contractions induced by low concentrations of norepinephrine and serotonin in human arteries.\textsuperscript{67} The potentiating effects of the peptides increase with age.\textsuperscript{68} This may contribute to the increased severity of vasospasm with age not only in the basilar artery but also in small arteries and intraparenchymal arterioles.

Another possible explanation is the impairment of endothelium-dependent relaxation. It has been well established that endothelium-dependent relaxations are less effective in cerebral vessels of old animals than in those of younger ones. In the present study, pulse pressure was significantly higher with age, suggesting that endothelium-dependent relaxations were impaired during aging, as previously shown in rabbits.\textsuperscript{69} In addition, it is well known that endothelium-dependent relaxations are also impaired in large arteries after SAH.\textsuperscript{48,49} These facts in combination may predispose the vessels to the impaired balance between endothelium-dependent contraction and relaxation, leading to the increased and continuous vasospasm of the basilar arteries with age.

In summary, this study demonstrates that aging renders the brain vulnerable to SAH, leading to augmented constricting responses of large conducting arteries, small arteries, and intraparenchymal arterioles with aging. Impaired reactivity to papaverine with aging may be a consequence of the early transition of the vessel to the papaverine-resistant chronic stage, in which functional and morphological changes take place. Even though further studies are necessary to elucidate how the mechanism of vasospasm is altered with age, our study suggests that future therapy should be focused on clinical vasospasm in the elderly not only in large arteries but especially in the microcirculation.

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