Subarachnoid Blood Causes Pial Arteriolar Constriction in Newborn Pigs

Helena Parfenova, PhD; Masaaki Shibata, PhD; Charles W. Leffler, PhD

Background and Purpose: The present study was designed to determine in newborn animals the delayed effect of subarachnoid blood on pial arteriolar diameter and eicosanoid concentrations in cortical periarachnoid fluid.

Methods: Forty-eight to 96 hours after subarachnoid blood installation, closed cranial windows were implanted over the cerebral area exposed to blood in anesthetized, artificially ventilated newborn piglets. All pial arterioles greater than 60 μm in diameter were measured, and cortical periarachnoid fluid was collected for the determination of eicosanoids.

Results: Subarachnoid blood resulted in a 20% to 30% decrease in the average diameter of pial arterioles exposed to blood for 48 to 96 hours, a decreased number of large pial arterioles (greater than 200 μm), and an increased number of small arterioles (60 to 100 μm). No changes in dilator prostanoids (prostacyclin as 6-keto-prostaglandin F₁α) and prostaglandin E₂ were detected. Concentrations of vasoconstrictor prostanoids in cortical cerebrospinal fluid increased. Thromboxane B₂ increased to 430±70 pg/mL, and prostaglandin F₁α increased to 1370±180 pg/mL compared with 250±20 and 860±70 pg/mL, respectively, in the control group. The concentration of peptidoleukotrienes increased to 400 to 600 pg/mL 72 to 96 hours after blood installation, while the level in the control group was less than 80 pg/mL.

Conclusions: The altered balance between vasodilator and vasoconstrictor eicosanoids could contribute to cerebral vasocostriction after subarachnoid blood installation in newborn pigs. (Stroke. 1993;24:1729-1734.)

Key Words • subarachnoid hemorrhage • vasoconstriction • pigs

Subarachnoid hemorrhage occurs in both mature and premature babies, while intraventricular/periventricular hemorrhages are very common in preterm babies and have involved with gestation age. Although considerable effort has been involved study of the effects of perivascular blood on adult cerebral vessels and on the etiology of perinatal cerebral hemorrhages, little study of the secondary effects of perivascular blood has been made in neonates. Such hemorrhages result in serious lifelong neurological complications.

Chronic vasospasm is one of the most serious complications in adult patients with subarachnoid hemorrhage, resulting in regional changes in cerebral blood flow, zones of cerebral ischemia, and infarction. Together with other delayed effects of hemorrhage, such as altered cerebral vascular responsiveness to physiological stimuli, these changes may play a role in the impairment of neurological function that often develops in patients with this injury.

Presently, there are no data on cerebral arteriolar diameter changes after exposure to extravascular blood in newborns. Arterioles on the cerebral surface (pial arterioles) are of great interest because, as major resistance vessels, they contribute to control of cerebral blood flow. Previous data from our laboratory demonstrate that chronic subarachnoid placement of autologous blood for 1 to 4 days in newborn pigs affects the exposed arterioles. These effects include altered responses to some vasoconstrictor neurotransmitters (norepinephrine) without substantial influence on responses to others (acetylcholine). Cerebrovascular dilatory responses to hypercapnia and hypotension are attenuated by chronic blood placement around pial vessels, while short exposure to blood (3 to 4 hours) does not change these vascular responses. Therefore, prolonged contact of pial arterioles to extravascular blood selectively attenuates cerebral vascular responses in newborn pigs. Whether alterations also occur in basal tone has not been examined.

Prostanoids appear to be involved in control of newborn pig cerebral microcirculation. Vasodilatory products of prostaglandin (PG) H synthase (prostacyclin, PGE₂) contribute to cerebral vasodilation induced by hypercapnia and hypotension. Other eicosanoids such as thromboxane (TX), PGF₂α and peptidoleukotrienes are potent constrictors of pial arterioles. Prostanoids can also modulate other vasoactive mechanisms in the newborn pig cerebral circulation (attenuating responses to norepinephrine or serving a permissive role in mediating responses to acetylcholine). Changes in cerebral eicosanoid metabolism could contribute to altered vascular reactivity after exposure to perivascular blood.
The present study was designed to determine in a newborn animal the effect of prolonged exposure to perivascular blood on pial arteriolar diameters and eicosanoid concentrations in cortical periarachnoid cerebrospinal fluid. (CSF)

Materials and Methods

The animal protocols used were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee, Memphis.

Perivascular Blood Installation

To investigate effects of extravascular blood contact on the vasculature, fresh autologous blood was placed in the subarachnoid space where it contacted pial blood vessels directly. Newborn pigs (age, 1 to 3 days; weight, 1.5 to 2.0 kg) were anesthetized with halothane and nitrous oxide. Using aseptic technique, a small burr hole was made in the skull over the frontal cortex. A 20-gauge Teflon catheter (Angiocath, Deseret Co, Sandy, Utah) containing a needle was used to pierce dura at an angle to the surface sufficient to prevent penetration into the brain. After removal of the needle, the tip of the catheter was advanced 2 cm caudally under the dura to the parietal cortex, and 2 to 3 mL of either fresh sterile nonheparinized blood (removed via direct puncture of the precava) (n=33) or sterile artificial cerebrospinal fluid (aCSF) (n=15, sham control) was slowly injected under the dura. The catheter was removed, the hole was filled with sterile bone wax, and the scalp was sutured. Piglets were treated with gentamicin after surgery. Following surgery, the piglets exhibited no apparent behavioral abnormalities.

Cranial Window Implantation

Pial arterioles were studied and cortical periarachnoid fluid collected using cranial windows implanted 48, 72, and 96 hours after blood placement. Piglets were initially anesthetized with ketamine hydrochloride (33 mg/kg IM) and acepromazine (3.3 mg/kg IM). Anesthesia was maintained with α-chloralose (30 to 50 mg/kg IV initially, supplemented with 5 mg/kg per hour IV). Catheters were inserted into the femoral artery for monitoring arterial blood gases, pH, and blood pressure and into the femoral vein for the injection of fluids. The animals were intubated and ventilated with air. Body temperature was maintained at 37 to 38°C. During the experiment arterial blood pressure was maintained within a range of 70 to 90 mm Hg; arterial blood gases were as follows: PaO₂, 86 to 90 mm Hg; PaCO₂, 33 to 36 mm Hg; and pH, 7.36 to 7.40.

The head was immobilized, and a hole 2 cm in diameter was cut in the skull over parietal cortex. The dura was cut and reflected over the bone. Clotted blood on the brain surface was removed when necessary, and the brain was flushed with aCSF. A stainless steel and glass cranial window was placed in the hole and fixed to the skull with dental acrylic. The space under the window was filled with aCSF through ports incorporated into the sides of the window. The aCSF composition was (in mmol/L): KCl 3.0; MgCl₂ 1.5; CaCl₂ 1.5; NaCl 132; urea 6.6; dextrose 3.7; and NaHCO₃ 24.6. The aCSF was warmed to 37°C and bubbled with gas mixture of 6% CO₂ and 6% O₂ in N₂ and showed pH, PCO₂, and PO₂ in the range of 7.33 to 7.44, 41 to 46 mm Hg, and 43 to 50 mm Hg, respectively. The volume of fluid directly under the window was ~500 µL and was contiguous with the periarachnoid space. Pial arteriolar diameter was measured with a videomicrocoumeter coupled to a television camera mounted on the microscope and a video monitor. Using the cranial window technique we were able to measure pial arteriolar diameters and sample cortical periarachnoid CSF for examination of cerebral production of prostanoids and leukotrienes.

Specific Protocols

To determine the effects of perivascular blood on pial arterioles and prostanoid and leukotriene production, all animals were divided into four groups: (1) sham control group (n=15), in which aCSF was injected subdurally; (2) 48 hours (n=14) after subdural blood injection; (3) 72 hours (n=10) after blood injection; and (4) 96 hours (n=9) after blood injection.

Before the experiment the space under the cranial window was flushed several times with aCSF, and animals were allowed to rest for 20 minutes. All pial arterioles greater than 60 µm in diameter under the window (typically five to eight) were measured in each animal. To determine diameter, measurements were taken every 2 minutes for 10 minutes. Cerebral production of prostanoids and leukotrienes was examined by analyzing cortical periarachnoid CSF collected from beneath the cranial window. CSF collection involved placing aCSF under the window for 10 minutes. At the end of the 10-minute period, 300 µL of CSF was sampled from under the window by slowly infusing aCSF into an inlet port of cranial window and allowing the cortical CSF to drip freely into a collection tube from an outlet port. Immediately after collection, cortical CSF samples were frozen and stored at −60°C before assays for prostanoids and leukotrienes.

Prostanoid Assays

Concentrations of 6-keto-PGF₁α (the hydrolysis product of prostacyclin), PGE₂, PGF₂α, and TXB₂ (the hydrolysis product of TXA₂) in the cortical CSF were determined by radioimmunoassay, as previously described in detail. Antibodies to the prostanoids were produced in rabbits immunized with prostanoids coupled to thyroglobulin using the mixed anhydride method. Cross-reactivities of our antibodies with other known, biologically relevant prostanoids were all minimal (less than 1%). Moreover, target ligands were not displaced from the antibodies by arachidonic acid (20 µg/mL); 5-hydroxyeicosatetraenoic acid (HETE) or 15-HETE (1 µg/mL); leukotriene (LT) B₄, LTC₄, LTD₄, or LTE₄ (5 µg/mL); or lipoxin A₄ or lipoxin B₄ (10 ng/mL). After incubation of CSF samples with the appropriate tritiated prostanoid and antibodies, the free tracer fraction was separated from the fraction bound to antibodies using dextran-coated charcoal. Standard curves were constructed with determination of second-order regression of tracer bound to antibody versus unlabeled prostanoid by the least-squares method. All unknowns were assayed at three dilutions, with parallelism between the unknown dilution curve and the standard curve required. The assay allowed analysis of prostanoid concentrations between 100 and 50,000 ng/mL.
**Effect of Intracranial Blood on Hemodynamics in Piglets**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MABP, mm Hg</th>
<th>Paco₂, mm Hg</th>
<th>Pao₂, mm Hg</th>
<th>pH</th>
</tr>
</thead>
<tbody>
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<td>Control</td>
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<td>80±3</td>
<td>35±1</td>
<td>90±1</td>
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</tr>
<tr>
<td>Blood</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>48 h</td>
<td>14</td>
<td>82±3</td>
<td>35±1</td>
<td>88±1</td>
<td>7.38±0.01</td>
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<tr>
<td>72 h</td>
<td>10</td>
<td>82±4</td>
<td>34±1</td>
<td>86±2</td>
<td>7.39±0.02</td>
</tr>
<tr>
<td>96 h</td>
<td>9</td>
<td>82±5</td>
<td>35±1</td>
<td>88±2</td>
<td>7.41±0.04</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MABP indicates mean arterial blood pressure.

**Leukotriene Assay**

Concentrations of peptidoleukotrienes in cortical CSF samples were measured using the AMI C₅/D₅/ E₅/F₅ [³H] RIA Kit (Advanced Magnetics Inc, Cambridge, Mass). Leukotrienes were extracted using ethyl acetate from acidified CSF samples (pH 3 to 4) before assay. Dried CSF extracts were dissolved in 0.01 mol/L phosphate buffer containing 0.1% gamma globulin, pH 8.5, and used for radioimmunoassay. Aliquots of CSF were incubated with monoclonal LTC₄/D₅/E₅/F₅ antibodies and LTC₄ tracer in 0.01 mol/L phosphate buffer (pH 8.5) for 2 hours at 25°C. Antibody-bound tracer was separated from the free tracer fraction using dextrancoated charcoal. The amount of LT C₅/D₅/E₅/F₅ in each sample was determined by interpolation from the standard curve. All assays were performed in duplicate. The assay allowed analysis of leukotriene concentrations between 80 and 20 000 pg/mL. Nondetectable leukotriene levels in CSF were given the value of 40 pg/mL for statistical purposes.

**Statistical Analysis**

Values are presented as mean±SEM of absolute values or as percent change from control values. Analysis of variance for repeated measurements was used for comparisons among different groups of animals. Fisher's protected least significant difference test was used for planned comparisons. An α-level of P<.05 was considered significant.

**Results**

**Changes in Pial Arteriolar Diameter After Subdural Blood Administration**

Arterial blood pressure, pH, and blood gases (Paco₂ and Pao₂) in animals treated with blood for 48 to 96 hours were not different from those in control animals (Table). The average pial arteriolar diameter in the control group of piglets was compared with the average diameter of pial arterioles exposed to blood 48, 72, and 96 hours after subdural blood administration. The average diameter of pial arterioles on the cerebral surface exposed to blood for 48 to 96 hours was significantly smaller than the diameter in the control group of piglets (Fig 1). The decreases in average pial arteriolar diameter were 21±3%, 27±5%, and 23±4% below the control level at 48, 72, and 96 hours of exposure, respectively.

The percentage of the largest pial arterioles (greater than 200 μm) was decreased from 13±3% in the control group to 2% to 4% after 48 to 96 hours' exposure to blood (Fig 2). The percentage of small arterioles (60 to 100 μm) was increased from 32±5% in the control group to 51±9% and 55±7% at 72 and 96 hours after blood installment, respectively (Fig 2). Therefore, there was a shift of distribution in pial arterioles toward smaller arterioles with transitional changes in intermediate groups after prolonged exposure to perivascular blood.

**Prostanoids and Leukotrienes in Cerebrospinal Fluid After Subdural Blood Administration**

No changes in the concentrations of 6-keto-PGF₁α or PGE₂ were detected in any of the experimental groups of piglets 48 to 96 hours after subdural blood administration when compared with the control group (Fig 3). In contrast, concentrations of all three vasoconstrictor eicosanoids measured (TxB₂, PGE₂, and LTC₄/D₅/E₅/F₅) were increased after installation of blood on the brain surface (Fig 4). The increases in TxB₂ and PGE₂ were relatively modest, with approximate doubling 72 and 96 hours after blood administration and with apparent but not significant increase at 48 hours. The increases in leukotrienes were much larger. Whereas CSF leukotriene levels in the control group of piglets were less than 80 pg/mL and no increase was noticed through the first 48 hours of blood exposure, peptidoleukotriene levels were increased to 410±200 and 611±340 pg/mL 72 and 96 hours, respectively, after blood administration.

**Discussion**

Our present data on pial arteriolar constriction after prolonged contact with blood are the first evidence of vasoconstriction following experimental subarachnoid...
hemorrhage in a newborn animal. The present study in newborn pigs demonstrates that the average diameter of pial arterioles over the cerebral surface that directly contacted installed autologous blood for 2 to 4 days was 20% to 30% less than the average diameter in the control group. In addition, there was a decrease in the largest pial arterioles (greater than 200 μm), whereas the fraction of the small arterioles (60 to 100 μm) was increased. The results clearly show a time-dependent shift of distribution pial arterioles toward smaller arterioles with transitional changes in intermediate groups. It appears that pial arteriolar constriction 48 to 96 hours after hemorrhage involves not only the largest arterioles but also medium-sized arterioles with diameter between 100 and 200 μm. The increasing number of arterioles with pial arteriolar diameter of less than 100 μm may suggest that the smallest pial arterioles are less affected by intracranial blood. These data indicate that pial arteriolar constriction occurred by 48 hours after subarachnoid hemorrhage and persisted for at least 96 hours.

Chronic vasospasm, which develops as a result of intracranial bleeding, is one of the most serious complications in adult patients with subarachnoid hemorrhage. In humans it is an important cause of morbidity in patients who survive the first 48 to 72 hours after subarachnoid hemorrhage and is also the major cause of delayed neurological deterioration in survivors. Most of the experimental studies of the secondary effects of intracranial blood have been performed in adult animal models: dogs, monkeys, and cats. Direct evidence of vasospasm is available for large cerebral arteries that can be visualized by angiography. Such angiographic evidence shows that chronic vasospasm of large cerebral arteries in adults develops 3 to 7 days after intracisternal or subarachnoid blood injection with a mean decrease in diameter to 15% to 70% of that in controls. Severe vasospasm produced significant decreases in regional cerebral blood flow in patients with subarachnoid hemorrhage and also resulted in changing brain metabolism. The mechanism of vasoconstriction that occurs as a result of intracranial bleeding has been intensively studied in adult animal models but still remains unclear. It has been suggested that the mechanism of blood-induced changes in the cerebral circulation represents a multifactorial process, which may include the direct effect of products released from the blood clot on exposed vessels. Eicosanoids are considered to be potentially involved compounds. This suggestion is based on evidence that prostanooids concentrations are elevated in the CSF, brain tissue, and blood vessels of animals with experimentally induced intracranial hemorrhage.

Prostanoids appear to be an integral component in the control of newborn pig cerebral microcirculation regulating basal vascular tone and vascular responses to...
physiological stimuli. Cerebral tissues and vessels produce prostanoids that can be detected in cortical periarachnoid CSF. Previous data demonstrate that intrathecal blood injection results in massive superoxide anion production via arachidonic acid metabolism in neonatal pigs. In the CSF of premature human infants suffering from periventricular/intraventricular hemorrhage, a wide variety of eicosanoids have been detected including TXB2, leukotrienes, and 6-keto-PGF1α, which are not detectable or greatly reduced in the CSF in patients without cerebral hemorrhages.

We have determined the concentrations of prostanoids as well as leukotrienes in CSF collected from control piglets and after intrathecal blood injection. We have found that concentrations of prostacyclin (as 6-keto-PGF1α) and PGE2, which are pial arteriolar dilators in newborn pigs, were unchanged in blood-treated animals. In contrast, concentrations of PGE2α and TXB2 were increased 72 and 96 hours after blood installation compared with the control group. PGE2α and TXA2 are potent vasoconstrictors of newborn pig cerebral arterioles.

We did not find significant changes in 6-keto-PGF1α levels in cortical CSF 48 to 96 hours after subarachnoid blood injection in newborn pigs. Therefore, it does not appear that chronic vasospasm in this model could be explained by a decrease in the formation of the vasodilator prostanoids. However, decreased sensitivity to vasodilator prostanoids cannot be excluded. In fact, we have recently reported that vasodilation in response to topical PGE2 is attenuated 4 days after subarachnoid injection of blood in newborn pigs. Significant increases in the biosynthesis of vasoconstrictor eicosanoids, thromboxane, and leukotrienes have been reported in most experimental models of intracranial hemorrhage in adults as well. Based on these data, it has been proposed that elevation of spasmogenic prostanoids is more likely to play a major role in the pathogenesis of chronic vasospasm. An attempt to prevent vasospasm from developing in adult patients with subarachnoid hemorrhages by inhibiting thromboxane synthetase resulted in significant clinical and angiographic improvement. Additionally, it has been reported that cyclooxygenase inhibitors, affecting both constrictor and dilator prostanoids, are beneficial in attenuating the cerebral vasospasm and changes in cerebral vascular reactivity that occur after blood administration.

Concentrations of peptidoleukotrienes, which are potent cerebral vasoconstrictors in newborn piglets, are also significantly increased 72 and 96 hours after autologous blood injection. Our data correlate with clinical findings that significant quantities of leukotrienes can be detected in the CSF of preterm babies suffering from intraventricular/periventricular hemorrhage, while leukotrienes are undetectable in the spinal fluid from normal babies. Leukotrienes are also significantly increased in the CSF of adult patients with aneurysmal subarachnoid hemorrhages. Increased synthesis of leukotrienes after intracranial blood injection has been demonstrated in adult rat and canine models. It was suggested that leukotrienes might be important etiologic factors in development of delayed vasospasm. In fact, angiographic evidence of chronic vasospasm in an adult canine model was reduced significantly by treatment with either AA-861, a selective inhibitor of 5-lipoxygenase, or ONO-1078, a potent leukotriene antagonist.

In conclusion, experimental subarachnoid hemorrhage in newborn pigs results in a 20% to 30% decrease of basal pial arteriolar diameter and increases in vasoconstrictor eicosanoids (TXA2 [as TXB2], PGE2α, and peptidoleukotrienes) in cortical CSF 48 to 96 hours after blood installation. CSF levels of vasodilator prostanoids (prostacyclin [as 6-keto-PGF1α] and PGE2) were not affected. We suggest that the elevation of spasmogenic eicosanoids may be involved in the pathogenesis of delayed cerebral vasoconstriction and potential secondary ischemia induced by cerebral hematoma in piglets. The altered balance between cortical vasodilator and vasoconstrictor eicosanoids might also affect cerebral vessels that have not been exposed to blood directly. Current data from our laboratory demonstrate that chronic effects of intraventricular blood include attenuation of pial arteriolar responses to prostanoid-dependent stimuli. Whether perivascular blood affects periventricular intraparenchymal arterioles similarly cannot be stated from the present experiments, but the potential for altered vascular responsiveness after periventricular hemorrhages certainly exists.

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References

Although the authors use the terms “subarachnoid hemorrhage (SAH)” and “vasospasm” throughout the accompanying article, one should realize that these terms usually indicate totally different things. As noted in the article, the vessels examined here are “important resistance” vessels, while those affected in clinical vaso- spasm—at least in adults—are conducting arteries. Whether conducting and resistance vessels respond to the same stimuli and have similar metabolism is doubtful.1,2 For instance, the reason that vasospasm is so common with aneurysmal SAH and so rare with SAH from a ruptured arteriovenous malformation remains totally unclear but may have something to do with the part of the cerebral circulation affected most. Moreover, the newborn circulation differs from the adult, eg, in the dependence on prostacyclin for vasodilation in response to hypercapnia.3 Thus, the present model may have more relevance to subependymal/intraventricular hemorrhage in the newborn than to “traditional” SAH. Nevertheless, the eicosanoids, particularly the leukotri- 

enes, may have a role in both conditions, possibly through their oxygen radical–inducing effects, as suggested by the authors. This then would suggest oxygen radical formation as a final common pathway from a number of mechanisms, which would be most amenable to treatment.

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**References**


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