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Research Report

Luminal platelet aggregates in functional deficits in parenchymal vessels after subarachnoid hemorrhage

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ABSTRACT

The pathophysiology of early ischemic injury after aneurysmal subarachnoid hemorrhage (SAH) is not understood. This study examined the acute effect of endovascular puncture-induced SAH on parenchymal vessel function in rat, using intravascular fluorescent tracers to assess flow and vascular permeability and immunostaining to assess structural integrity and to visualize platelet aggregates. In sham-operated animals, vessels were well filled with tracer administered 10 s before sacrifice, and parenchymal escape of tracer was rare. At ten minutes and three hours after hemorrhage, patches of poor vascular filling were distributed throughout the forebrain. Close examination of these regions revealed short segments of narrowed diameter along many profiles. Most vascular profiles with reduced perfusion contained platelet aggregates and in addition showed focal loss of collagen IV, a principal component of basal lamina. In contrast, vessels were well filled at 24 h post-hemorrhage, indicating that vascular perfusion had recovered. Parenchymal escape of intravascular tracer was detected at 10 min post-hemorrhage and later as plumes of fluorescence emanating into parenchyma from restricted microvascular foci. These data demonstrate that parenchymal microvessels are compromised in function by 10 min after SAH and identify focal microvascular constriction and local accumulation of luminal platelet aggregates as potential initiators of that compromise.

1. Introduction

Aneurysmal subarachnoid hemorrhage (SAH) is accompanied by early cerebral ischemic injury and high 48 hour mortality (Bederson et al., 1998; Gewirtz et al., 1999; Stoltenburg-Didinger and Schwartz, 1987). The mechanisms of early ischemic injury are poorly understood and specific treatment options are limited.

Recently attention has turned to the cerebral parenchymal vessel as a possible site of early ischemic injury after SAH (Ishikawa et al., 2009; Scholler et al., 2007; Sehba and Bederson, 2006; Yatsushige et al., 2007). Structural changes in parenchy-

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Abbreviations: SAH, subarachnoid hemorrhage; BP, blood pressure; ICP, intracranial pressure; CBF, cerebral blood flow

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mal microvessels sufficient to affect their function are found as early as 10 min after SAH. These include microvascular constriction, focal accumulation of luminal platelet aggregates (Sehba et al., 2005), detachment of endothelium lining, the appearance of gaps in the collagen IV-immunoreactive basal membrane, and the transmigration of platelets or platelet aggregates into the brain parenchyma (Friedrich et al., 2010; Sehba et al., 2004, 2005, 2007). These observations indicate that rapid and dramatic changes in the structure of the brain microvessels occur following SAH, but do not directly address the relationship between these events and perfusion and permeability. The current study combines intravascular tracer analysis with immunostaining and three-dimensional visualization to visualize disturbances in perfusion and permeability and to relate them to structural changes described previously.

2. Results

2.1. Microvascular perfusion

2.1.1. FITC-dextran vascular labeling

Fixable FITC-dextran was injected intrafemorally 10 s before sacrifice. In sham-operated controls, the tracer filled the cerebral vasculature (Fig. 1A) and labeled vessels were uniformly distributed across the two hemispheres and across the brain regions (basal, frontal, and convexity cortex, and striatum). The right-left symmetry of labeling demonstrates that brief (unilateral) introduction of the monofilament into the ICA, without induction of hemorrhage, does not cause perfusion deficits.

By contrast, in SAH animals pale and bright vascular patches of poor and well filled fluorescent label were present (Fig. 1A). These patches exhibited no specific anatomical pattern and were present in both hemispheres and in all the brain regions. At high magnification pale patches showed little vascular fluorescence, indicating substantially reduced rate of perfusion. In contrast, a substantial amount of fluorescence was present in the brighter, better-perfused patches (Fig. 1B). The patches of poor perfusion were evident at 10 min post-hemorrhage (not shown) and prevalent at 3 h post-hemorrhage (Fig. 1A). At 24 h post-hemorrhage, vessel filling had substantially improved (Fig. 1A). These patches of poor perfusion were absent at all survival intervals from sham-operated specimens, indicating that impaired perfusion resulted neither from surgery without hemorrhage nor from the procedure used for injecting vascular tracer but rather from the subarachnoid hemorrhage and its sequellae.

We determined the area fraction of FITC-dextran positive vascular profiles in our images as a rough quantitative index of vascular perfusion. This index was significantly reduced at 10 min and 3 h post-hemorrhage and increased at 24 h in comparison to sham-operated animals (Fig. 1B). In addition, we determined the average vascular diameter in sham and SAH animals (Fig. 1C). The data showed no significant change in the vascular diameter at 10 min, a small but significant decrease at 3 h ($P=0.003$), and small but significant increase (dilation) at 24 h post-hemorrhage.

2.1.2. FITC-dextran and platelet immunostaining

To assess the relationship between vascular perfusion and luminal platelet aggregates, we immunostained the brain sections of animals sacrificed 3 h after SAH for platelet-specific antigen. As reported previously, a large number of parenchymal vessels contained luminal platelet aggregates (Sehba et al., 2005). Under low magnification these aggregates appeared as red dots in otherwise green fluorescent vascular profiles. In high magnification two kinds of vascular segments could be identified; segments that contained little FITC-dextran label and were filled with platelet aggregates; indicating mechanical obstruction in flow and segments with decreased diameter filled with platelet aggregates and FITC-dextran; indicating local constriction (Fig. 2). In addition, many continuous FITC-dextran label vascular profiles appeared to be interrupted such that they terminated for a brief (approximately 5 μm) distance and then continued. Platelet staining was always found at the edges of the interrupted segments (Fig. 2).

2.2. Microvascular permeability

2.2.1. FITC-albumin extravasation

To assess blood–brain barrier function, we injected animals intrafemorally with FITC-albumin and sacrificed them 15 min later by vascular perfusion with saline followed by a formaldehyde fixative. The perfusion cleared most of the intravascular FITC-albumin, leaving extravascular FITC which marked sites where albumin had escaped from the vasculature. Sections were also immunostained for collagen IV to visualize the microvascular basal lamina. Little extravasation was evident in sham-operated animals, indicating that the blood–brain barrier was intact after sham surgery. By contrast in SAH animals approximately 20% collagen IV positive parenchymal vessel profiles were associated with small accumulations of extravascular FITC-albumin. These foci of extravasation were visible in 10 min, 3 h and 24 h post-hemorrhage specimens, in all the brain regions and in both hemispheres. High magnification, 3D confocal microscopy showed small windows in collagen IV immunofluorescence near the vascular sites of extravasation (Fig. 3A). Much of the extravasated FITC-albumin was located in the parenchymal cells; it indicates active phagocytosis (Fig. 3B). To quantitate extravasation, the number of vessels with FITC-albumin extravasation was counted. A substantially greater number of vessels exhibited FITC-albumin extravasation at 10 min to 24 h post-hemorrhage as compared to time matched sham cohorts (Fig. 3C).

In addition to parenchyma, FITC-albumin fluorescence was also present in the lumen of vessels. In higher magnification vascular fluorescence was found to be located within luminal cells (Fig. 3B). The number of these vascular cells decreased with time after SAH. No fluorescent cells were found in parenchymal vessels of sham-operated animals.

2.2.2. FITC-albumin and platelet immunostaining

Most vessels with FITC-albumin extravasation contained luminal platelet aggregates. In some cases platelets appeared to be extravasating along with FITC-albumin into the parenchyma via an apparent shared vascular exit site (Fig. 4).