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Research Report

Characterization of microvascular basal lamina damage and blood–brain barrier dysfunction following subarachnoid hemorrhage in rats

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ABSTRACT

Vasogenic brain edema is one of the major determinants for mortality following subarachnoid hemorrhage (SAH). Although the formation of vasogenic brain edema occurs on the microvascular level by opening of endothelial tight junctions and disruption of the basal lamina, microvascular changes following experimental SAH are poorly characterized. The aim of the present study was therefore to investigate the time course of blood–brain barrier (BBB) dysfunction and basal lamina damage following SAH as a basis for the better understanding of the pathophysiology of SAH. SAH was induced in Sprague–Dawley rats by an endovascular filament. Animals were sacrificed 6, 24, 48, and 72 h thereafter ($n=9$ per group). Microvascular basal lamina damage was quantified by collagen type IV immunostaining. Western blotting was used to quantify collagen IV protein content and bovine serum albumin (BSA) extravasation as a measure for basal lamina damage and blood–brain barrier disruption, respectively. BSA Western blot revealed significant ($p<0.05$) BBB opening in the cerebral cortex ipsilateral to the hemorrhage beginning 6 h and peaking 48 h after SAH. Significant ($p<0.05$) basal lamina damage occurred with gradual increase from 24 to 72 h. Basal lamina damage correlated significantly with BBB dysfunction ($r=-0.63$; $p=0.0001$). Microvascular damage as documented by collagen IV degradation and albumin extravasation is a long lasting and ongoing process following SAH. Due to its delayed manner microvascular damage may be prone for therapeutic interventions. However, further investigations are needed to determine the molecular mechanisms responsible for basal lamina degradation and hence damage of the microvasculature following SAH.

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1. Introduction

The majority of subarachnoid hemorrhages (SAH), i.e. 85%, are caused by ruptured aneurysms (Drake, 1971, 1977). The initial hemorrhage and re-bleedings account for the high morbidity and mortality in the early phase after SAH. The major consequence of the initial bleeding is the rise of intracranial pressure (ICP) followed by a decrease of cerebral blood flow (CBF). The subsequent global ischemia damages predominantly the cerebral cortex (Crompton, 1964; Neil-Dwyer et al., 1994) and causes severe neurological deficits, and a high mortality (Davis et al., 1992; Matsuda et al., 1990; Meyer et al., 1983; Mickey et al., 1984). The detrimental post-ictal ICP increase occurs mainly due to the space-occupying effect of the hematoma, the acute vasoparalysis with consecutive increase of intracranial blood volume, and the rapidly developing cerebral edema (Claassen et al., 2002; Grote and Hassler, 1988; Johshita et al., 1990; Kuyama et al., 1984). Furthermore, Claassen and coworkers (2002) identified brain edema formation as an independent risk factor for mortality and poor outcome after SAH. As demonstrated by several studies disruption of the blood–brain barrier (BBB) was the primarily cause of SAH-induced brain edema (Doczi, 1985; Doczi et al., 1986a,b; Germano et al., 2000; Sasaki et al., 1986; Trojanowski, 1982). As shown by us in a recent study brain edema peaks 24 h following experimental SAH in rats and stays elevated for up to 48 h (Thal et al., 2005).

Despite the clinical importance of early brain edema formation following SAH, the mechanisms involved, particularly on the microvascular level, are poorly understood. Hence, the present study was designed to elucidate extent and time course of microvascular basal lamina injury and its relationship to BBB dysfunction after SAH in rats.

2. Results

2.1. *Mortality*

No mortality was observed in sham operated rats. From the animals subjected to SAH nine died immediately after vessel perforation or had to be sacrificed because of persistent coma after cessation of anesthesia. Mortality increased with the time of survival after SAH (6%, 17%, 25%, and 32% mortality in the animals surviving SAH for 6, 24, 48, and 72 h, respectively).

2.2. *Physiological parameters and monitoring parameters*

Experimental groups did not differ with regard to pre- and post-hemorrhagic arterial blood gases, serum glucose, or serum lactate (Table 1, $p<0.05$; one-way ANOVA followed by Holm–Sidak method) as well as with regard to brain and rectal temperature. There was also no significant difference in pre-hemorrhagic (Sham: 73±14 mmHg, SAH 6 h: 75±15 mmHg, SAH 24 h: 79±6 mmHg, SAH 48 h: 79±15 mmHg, SAH 72 h: 74±14 mmHg) and post-hemorrhagic MABP (Sham: 69±15 mmHg, SAH 6 h: 80±19 mmHg, SAH 24 h: 81±12 mmHg, SAH 48 h: 79±15 mmHg, SAH 72 h: 73±20 mmHg).

Table 1 – Intraoperative physiological variables

Time	−60 min					−15 min					+15 min				
Group	Sham	SAH 6 h	SAH 24 h	SAH 48 h	SAH 72 h	Sham	SAH 6 h	SAH 24 h	SAH 48 h	SAH 72 h	Sham	SAH 6 h	SAH 24 h	SAH 48 h	SAH 72 h
PH	7.37±0.16	7.42±0.07	7.41±0.11	7.36±0.05	7.37±0.005	7.41±0.09	7.39±0.06	7.38±0.05	7.39±0.04	7.38±0.05	7.39±0.06	7.36±0.05	7.34±0.06	7.36±0.04	7.36±0.05
pO_2 [mmHg]	115.7±12.1	102.3±23.3	111.4±13.2	104.5±22.2	97.3±17.1	111.4±15.3	95.7±17.1	107.8±16.9	109.5±17.6	100.4±18.4	112.1±16.9	94.9±22.8	100.7±14.5	100.2±24.5	88.5±18.0
pCO_2 [mmHg]	35.1±10.7	32.7±8.5	35.3±14.5	37.6±7.6	38.9±8.1	36.1±10.8	34.0±10.7	36.8±8.9	38.0±4.5	37.8±7.2	33.1±8.1	39.2±5.9	40.9±7.4	41.9±5.2	42.2±8.8
Glucose [mg/dl]	109.7±29.1	99.8±17.8	114.2±25.2	110.3±20.6	111.2±24.5	100.6±19.0	90.1±21.2	98.1±18.8	104.9±9.6	106.8±20.2	97.7±36.6	97.7±26.4	100.2±23.6	106.8±6.4	116.0±17.8
Lactate [mmol/l]	1.0±0.4	1.1±*0.3	1.0±§0.4	1.2±0.4	1.2±0.5	0.9±0.4	0.7±0.2	0.7±0.2	0.9±0.2	0.9±0.5	0.8±0.4	0.7±0.1	0.7±0.2	1.1±0.7	0.9±0.3

Physiological variables 60 and 51 minutes (−60 min, −15 min) prior to SAH induction and 15 minutes (+15 min) after SAH induction. There is no significant difference in physiological variables between groups. Values are mean±SD for n=9 in each group. *$P<0.05$ vs. −15/+15 min, §$P<0.05$ vs. −15 min (one-way ANOVA, Holm-Sidak method).